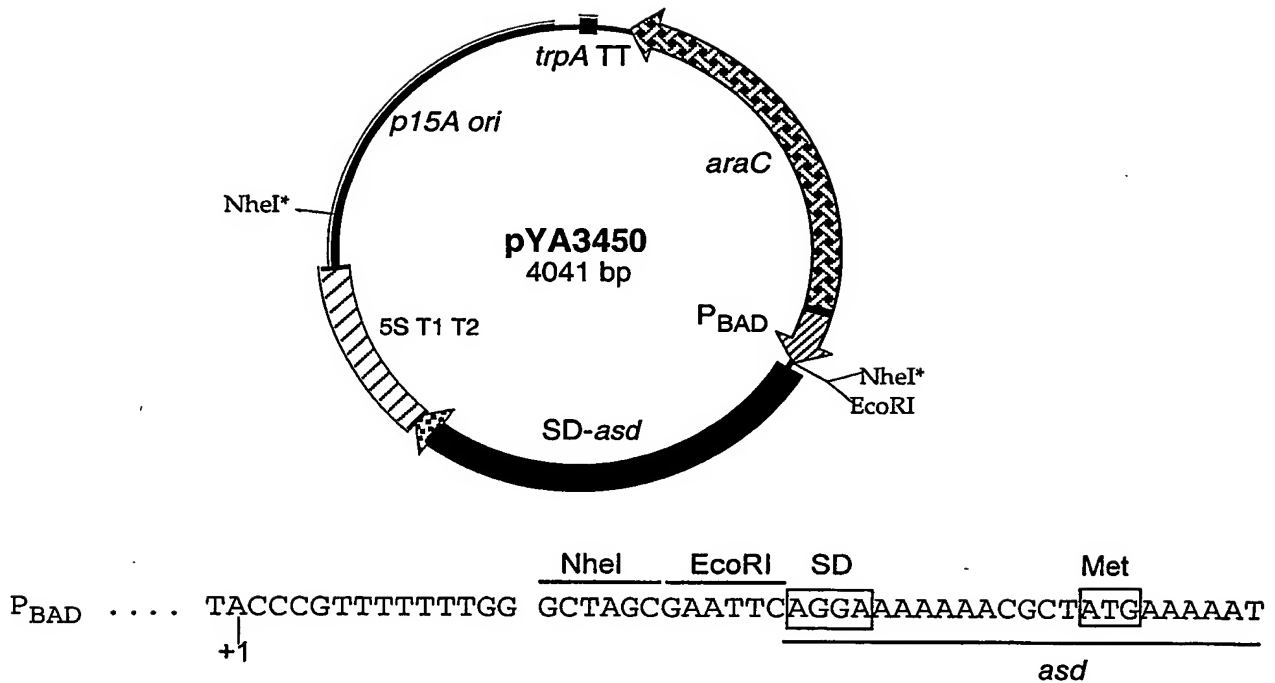


FIGURE 1. pYA3450



pYA3450 is an *araC* P_{BAD} *asd* vector derived from pYA247. Around 35 bp (the number was calculated based on the map of pYA247 from MEGAN) unnecessary sequence between P_{BAD} and *asd* was deleted by replacing the original *asd* gene with a 1,100 bp EcoRI-HindIII PCR product derived from the *asd* gene of pYA292. The PCR product contains the *asd* gene from 300 to 1420 bp including the SD sequence. The pYA247 was cut with EcoRI and HindIII and the 2.9 kb fragment was isolated and ligated with the 1.1 kb PCR product of *asd*.

FIGURE 2A. pYA3530

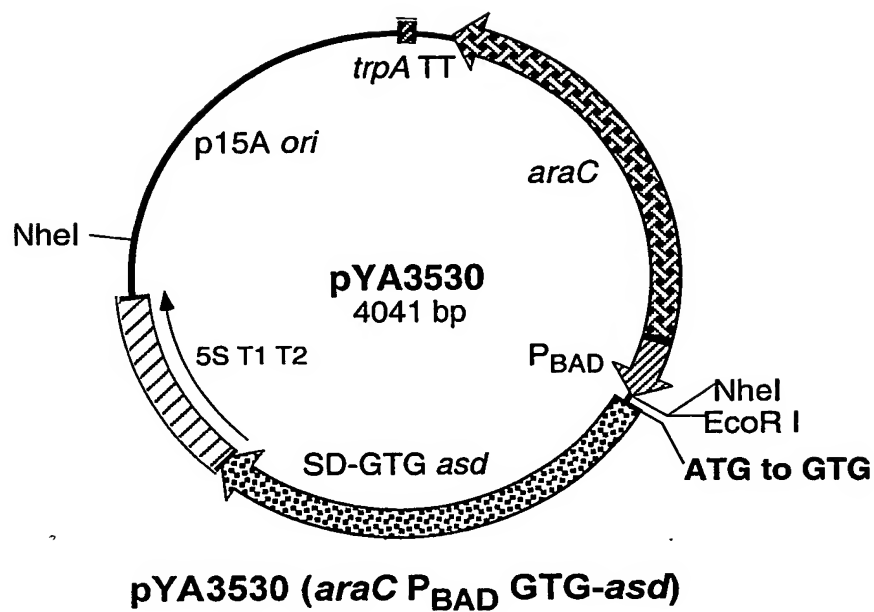
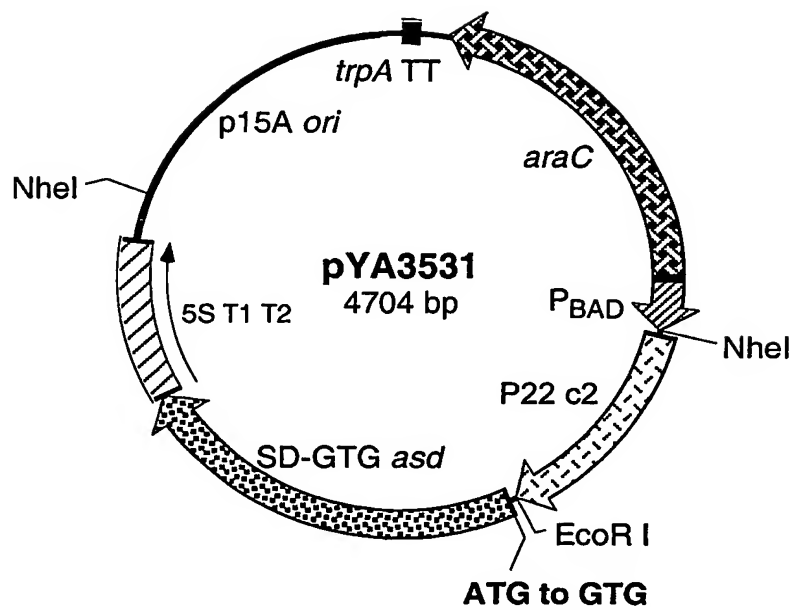


FIGURE 2B. pYA3531



pYA3531 (*araC* *P_{BAD}* *P22 c2* *GTG-asd*)

FIGURE 3. Growth of $\chi 8645 \Delta P_{murA7}::araC P_{BAD} murA$ in 1% Rodent Chow, 1% Chicken Feed and 1% Chicken Breast Meat Broth +/- 0.5% Arabinose

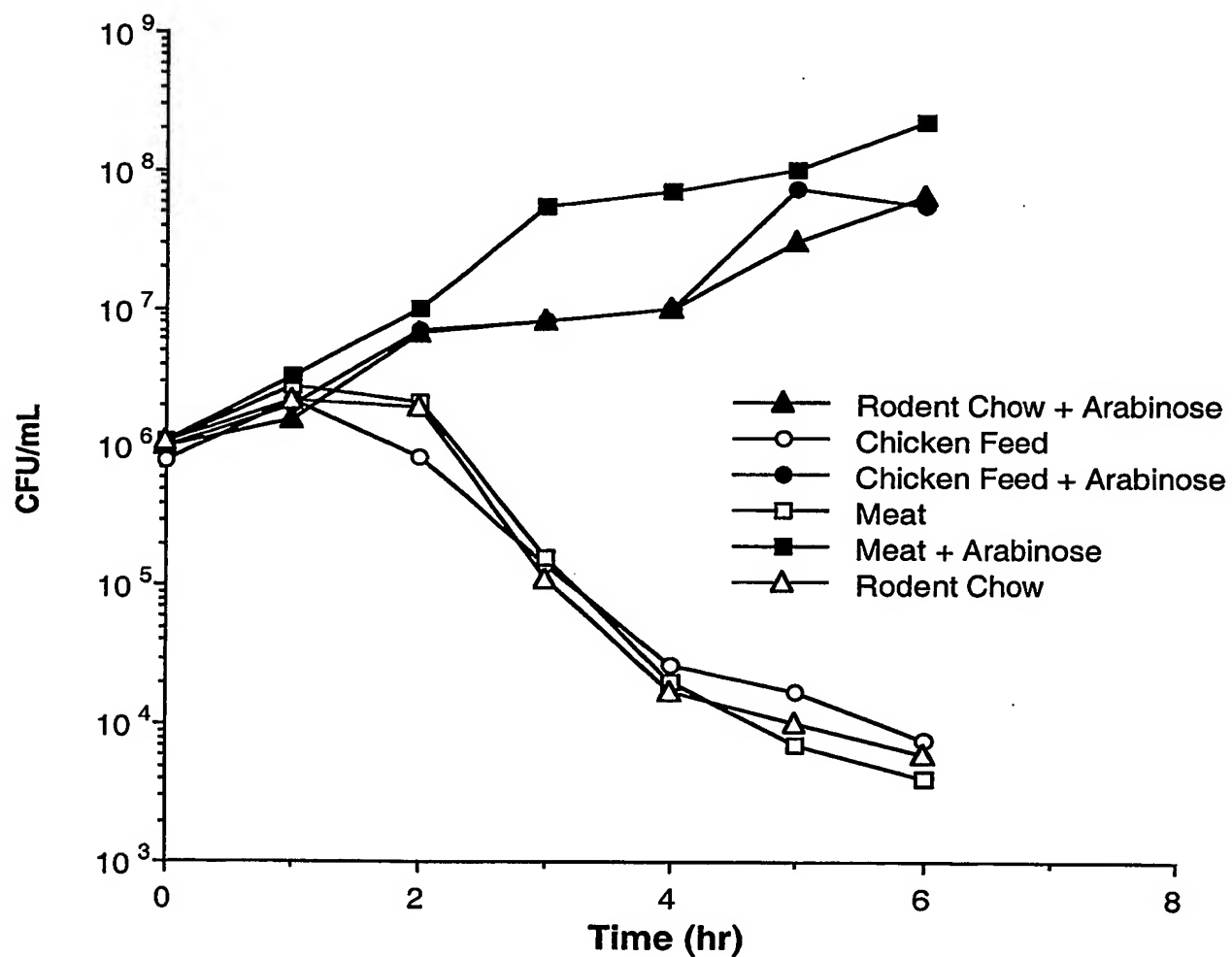
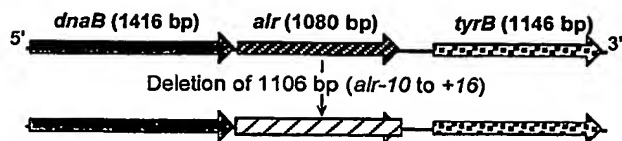
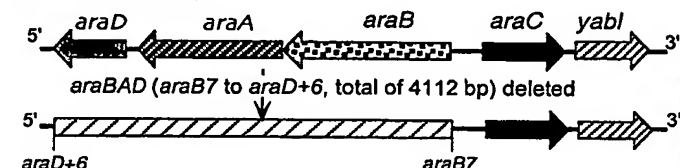
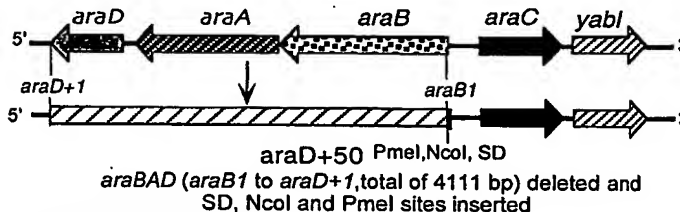
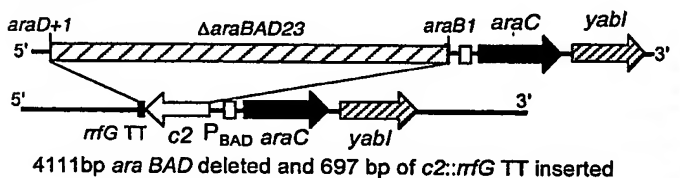
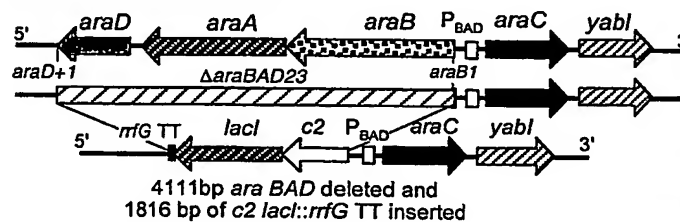
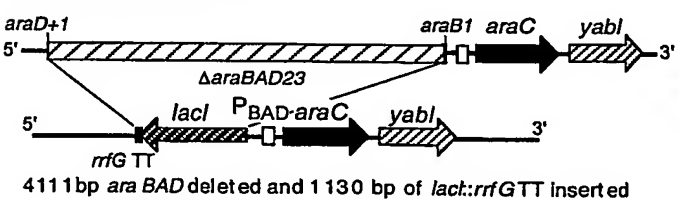
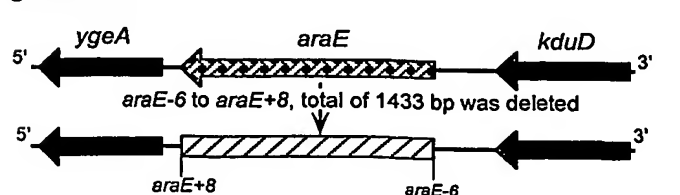
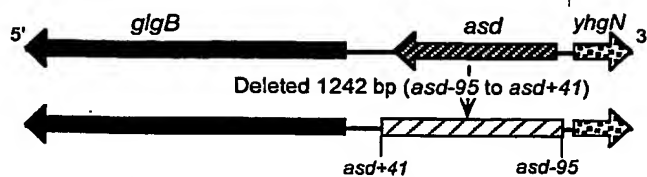
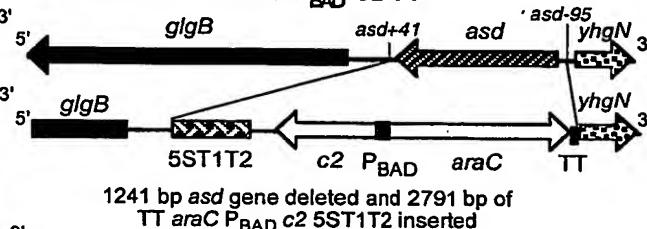
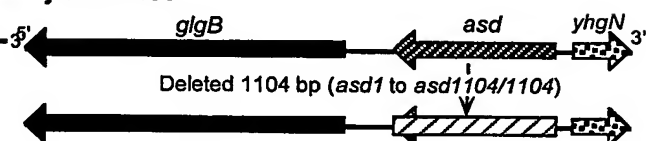
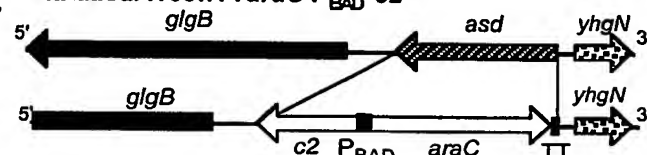
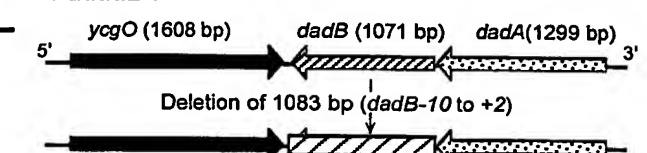
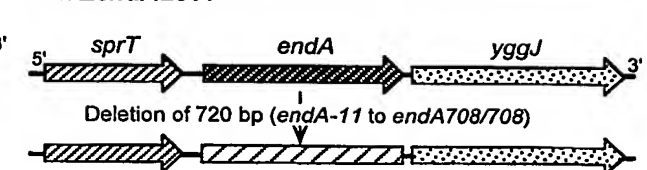
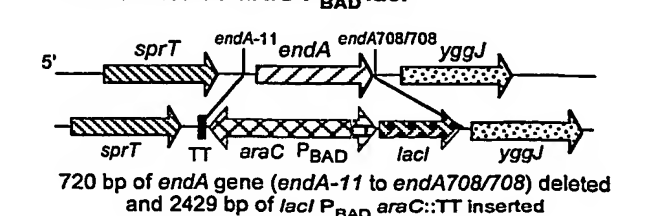


FIGURE 4. Structure of deletion and deletion-insertion mutations

a. $\Delta alr-3$ b. $\Delta araBAD1923$ c. $\Delta araBAD23$ d. $\Delta araBAD23$ c2::TTe. $\Delta araBAD23$ c2 *lacI*::TTf. $\Delta araBAD23$ *lacI*::TTg. $\Delta araE25$ h. $\Delta asdA16$ i. $\Delta asdA19::TT araC P_{BAD} c2$ TTj. $\Delta asdA33$ k. $\Delta asdA183::TT araC P_{BAD} c2$ l. $\Delta dadB4$ m. $\Delta endA2311$ n. $\Delta endA23::TT araC P_{BAD} lacI$ 

▨: deleted region

FIGURE 4. continued

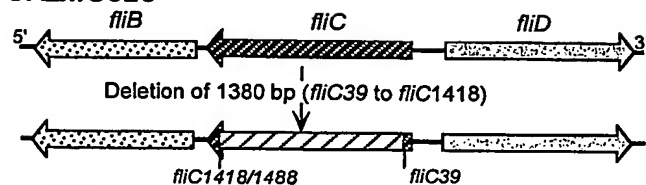
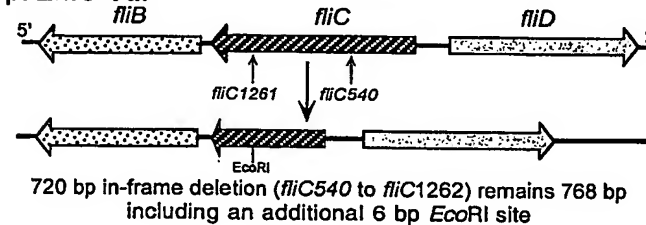
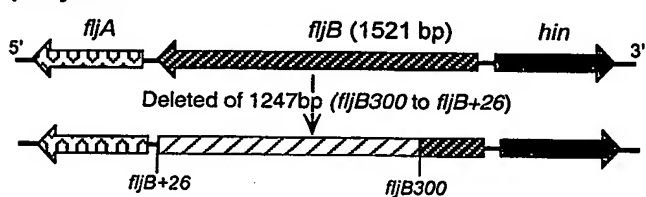
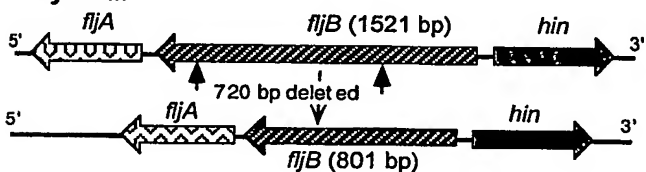
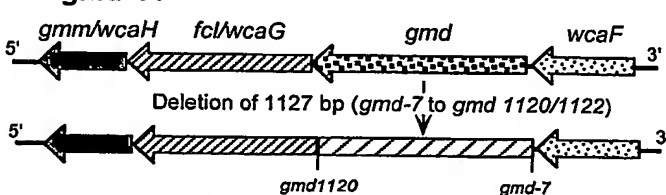
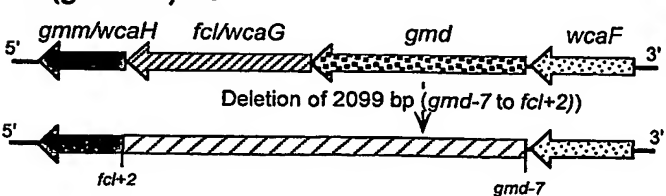
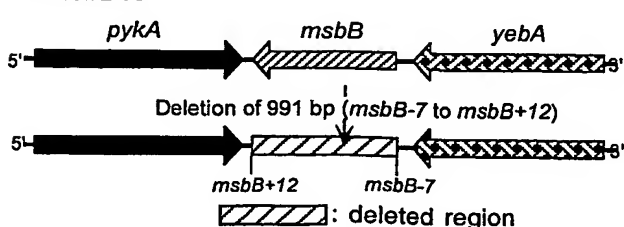
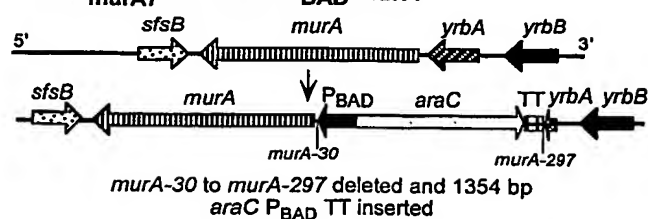
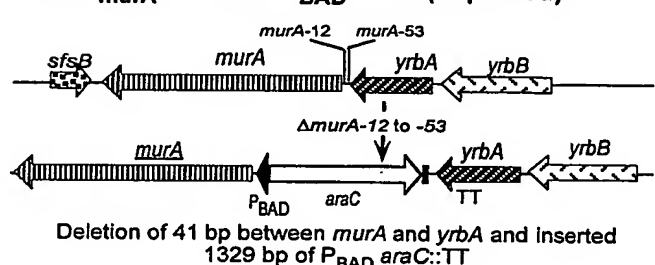
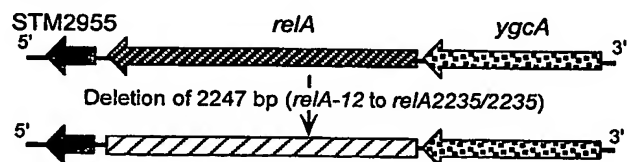
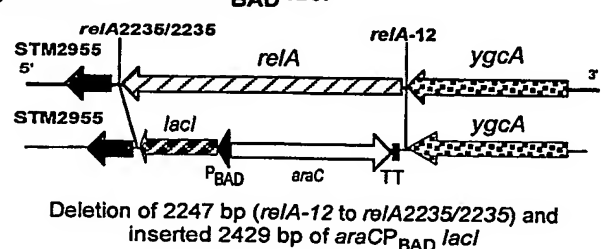
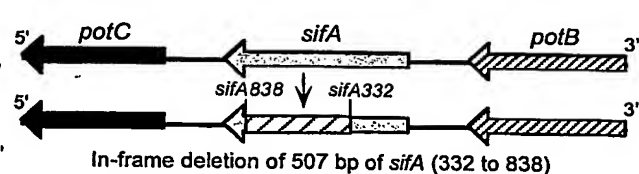
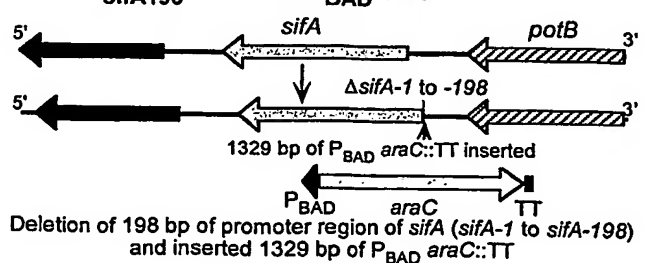
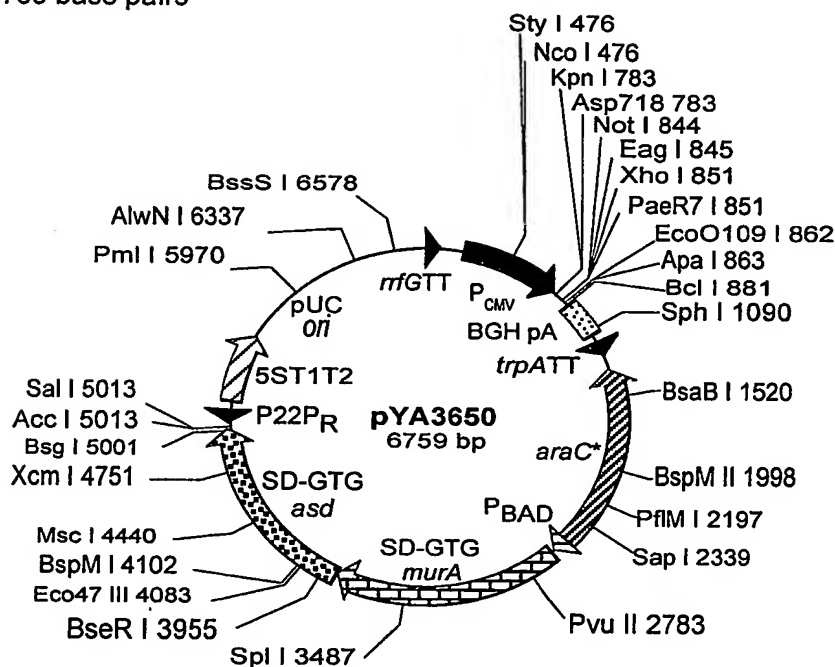
o. $\Delta fliC825$ p. $\Delta fliC$ -Varq. $\Delta fljB217$ r. $\Delta fljB$ -Vars. $\Delta gmd-11$ t. $\Delta(gmd-fcl)-26$ u. $\Delta msbB48$ v. $\Delta P_{murA7}::TT$ *araC* P_{BAD} *murA*w. $\Delta P_{murA}::TT$ *araC* P_{BAD} *murA* (Improved)x. $\Delta relA1123$ y. $\Delta relA::TT$ *araC* P_{BAD} *lacI*z. $\Delta sifA26$ aa. $\Delta P_{sifA196}::TT$ *araC* P_{BAD} *sifA*

FIGURE 5A. DNA vaccine vector pYA3650

6,759 base pairs



DNA vaccine vector pYA3650 designed to express eukaryotic DNA from human cytomegalovirus immediate early gene promoter.

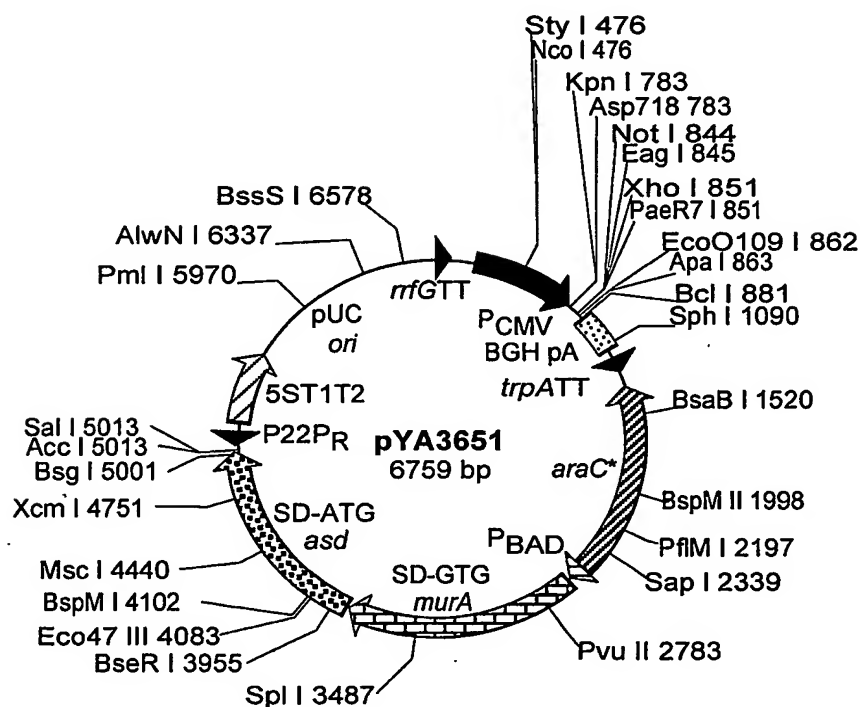
pYA3650 contains the immediate early gene promoter / enhancer from CMV, the Bovine growth hormone (BGH) gene polyadenylation signal, transcriptional termination sequence to enhance mRNA stability, a regulatable activator-promoter complex controlling the in vitro/in vivo expression of two genetically modified genes specifying enzymes necessary for synthesis of the rigid layer of the bacterial cell wall, a regulated synthesis of antisense RNA to completely turn off in vivo translation of mRNA encoded by the two genes whose products are necessary for synthesis of the rigid layer of the bacterial cell wall, and a replicon necessary for replication in bacteria but not in eukaryotic cells.

The exact locations are: *rfGTT* 36-100, *trpATT* 1243-1271, SD-GTG *murA* 2595/3868, 5ST1T2 5178/5620, P_{CMV} 202-789, *araC* 2497-1377, SD-GTG *asd*, 3889/4995, pUC *ori* 6081/6754, BGH pA 888/1118, P_{BAD} 2498-2582 and P22P_R 5106-5027.

The map shows the location of sites for enzymes that cleave the molecule once.

FIGURE 5B. DNA vaccine vector pYA3651

6,759 base pairs



DNA vaccine vector pYA3651 possesses SD-ATG-*asd* designed to increase translational level of Asd mRNA compared to pYA3650.

The exact locations are: *rrfGTT* 36-100, *trpATT* 1243-1271, SD-GTG *murA* 2595/3868, 5ST1T2 5178/5620, P_{CMV} 202-789, *araC** 2497-1377, SD-ATG *asd*, 3889/4995, pUC *ori* 6081/6754, BGH pA 888/1118, P_{BAD} 2498-2582 and P22P_R 5106-5027.

The map shows the location of sites for enzymes that cleave the molecule once.

FIGURE 6. Suicide Vectors

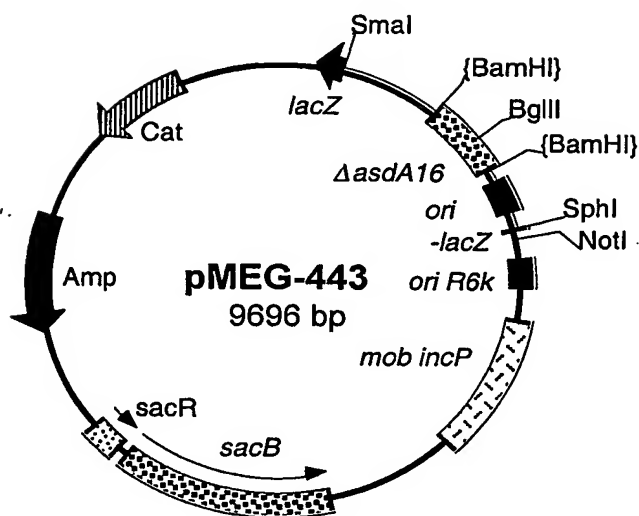
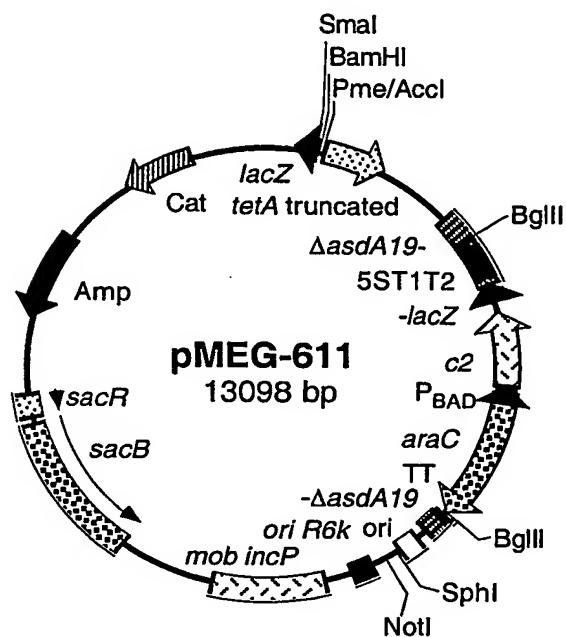
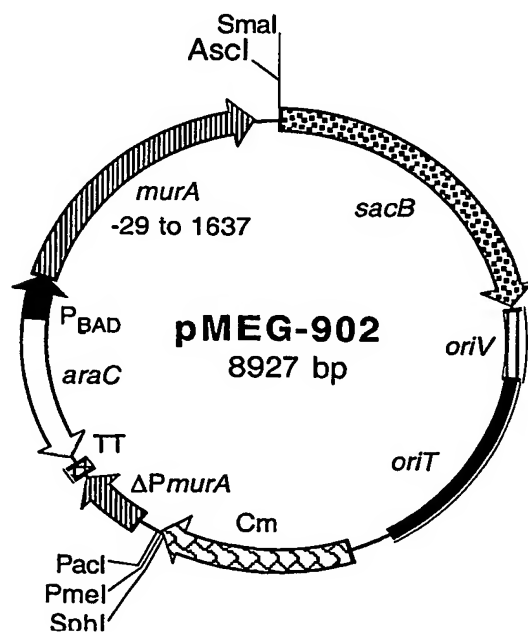
A. $\Delta asdA16$ B. $\Delta asdA19::TT araC P_{BAD} c2$ C. $\Delta P_{mur7}::TT araC P_{BAD} murA$ 

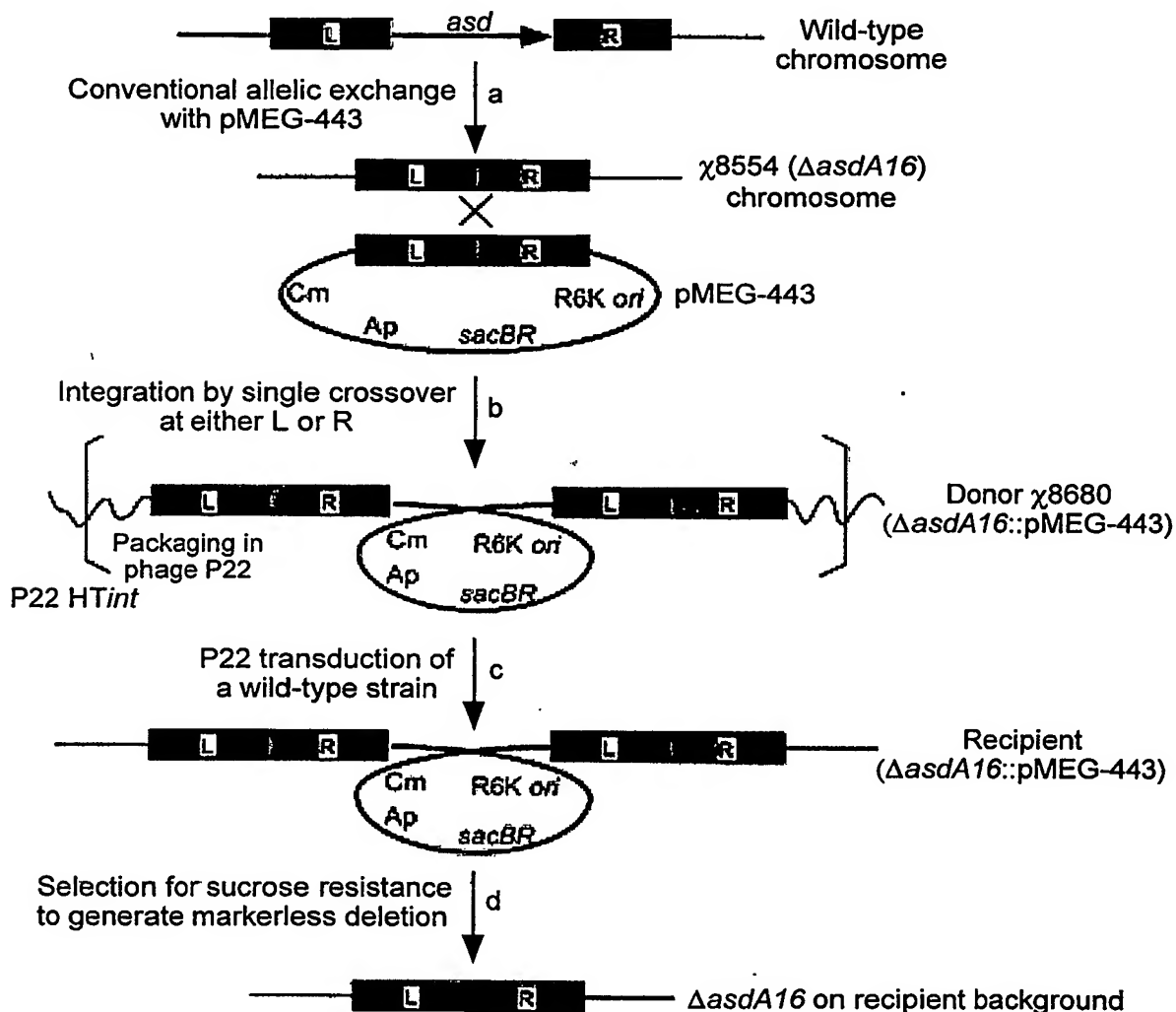
FIGURE 7. Transductional Method of Moving Unmarked Mutations

Illustration of overall processes for transfer of the Δ *asdA16* mutation. Black boxes and gray boxes represent cloned 5' (left [L]) and 3' (right [R]) flanking regions, respectively, of the *asd* gene. (Step a) Using the recombinant suicide plasmid pMEG-443, a Δ *asdA16* mutant was generated by the routine allelic-exchange method. (Step b) Plasmid pMEG-443 was integrated into the chromosome of the χ 8554 (Δ *asdA16*) strain by single-crossover insertion. (Step c) Phage P22HTint was propagated on the donor strain χ 8680 (Δ *asdA16*::pMEG-443). The Δ *asdA16*::pMEG-443 complex was transduced to a wild-type recipient strain, and transductants were selected based on the plasmid-encoded antibiotic resistance markers. (Step d) Excision of the plasmid by homologous recombination between duplicated regions was selected for by using the plasmid-carried *sacB* counterselection system to generate the unmarked deletion mutation.

FIGURE 8. Construction of suicide vectors $\Delta asdA33$ and $\Delta asdA183::TT araC P_{BAD} c2$ for use in *S. typhi* and *S. paratyphi* A

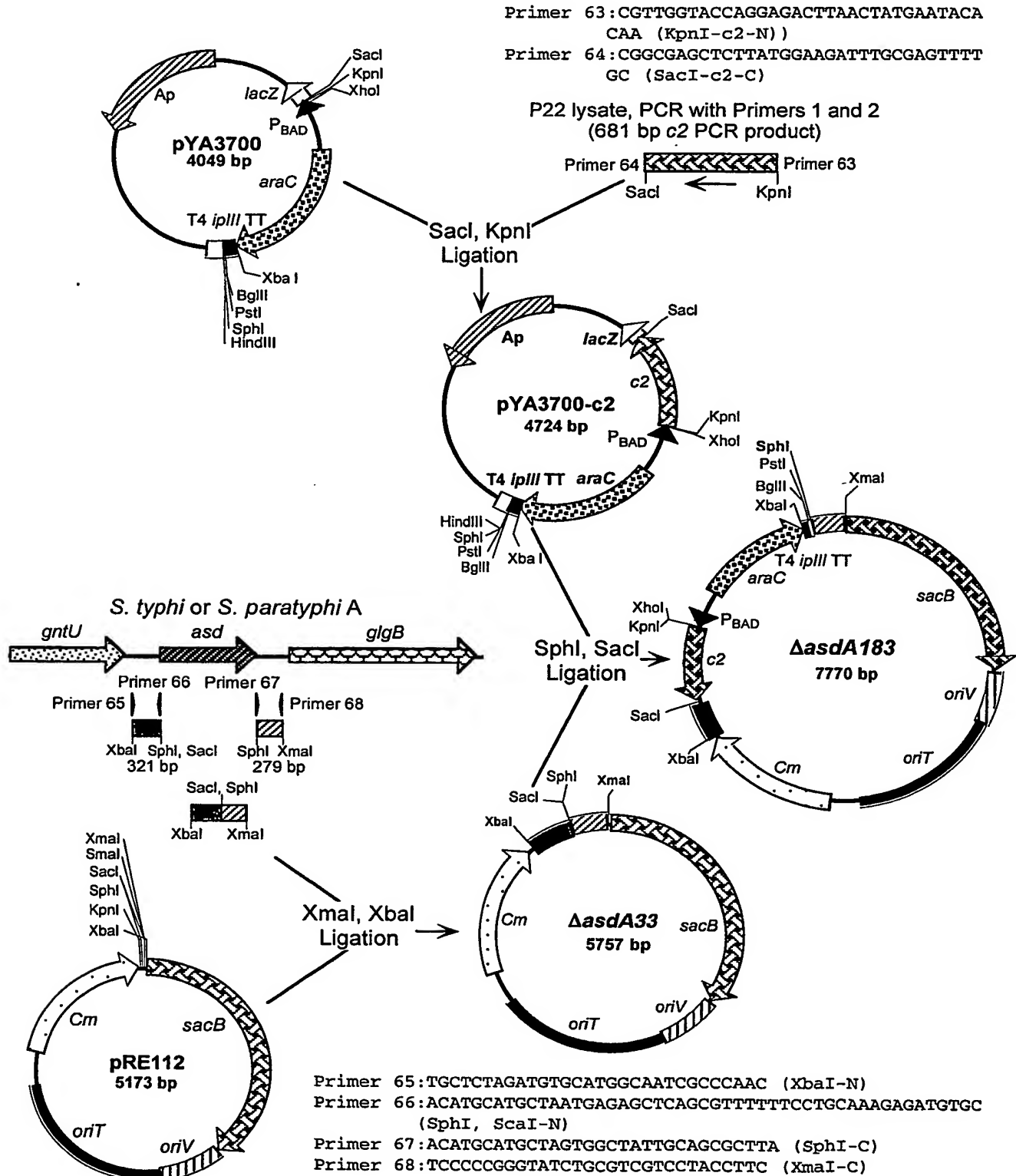
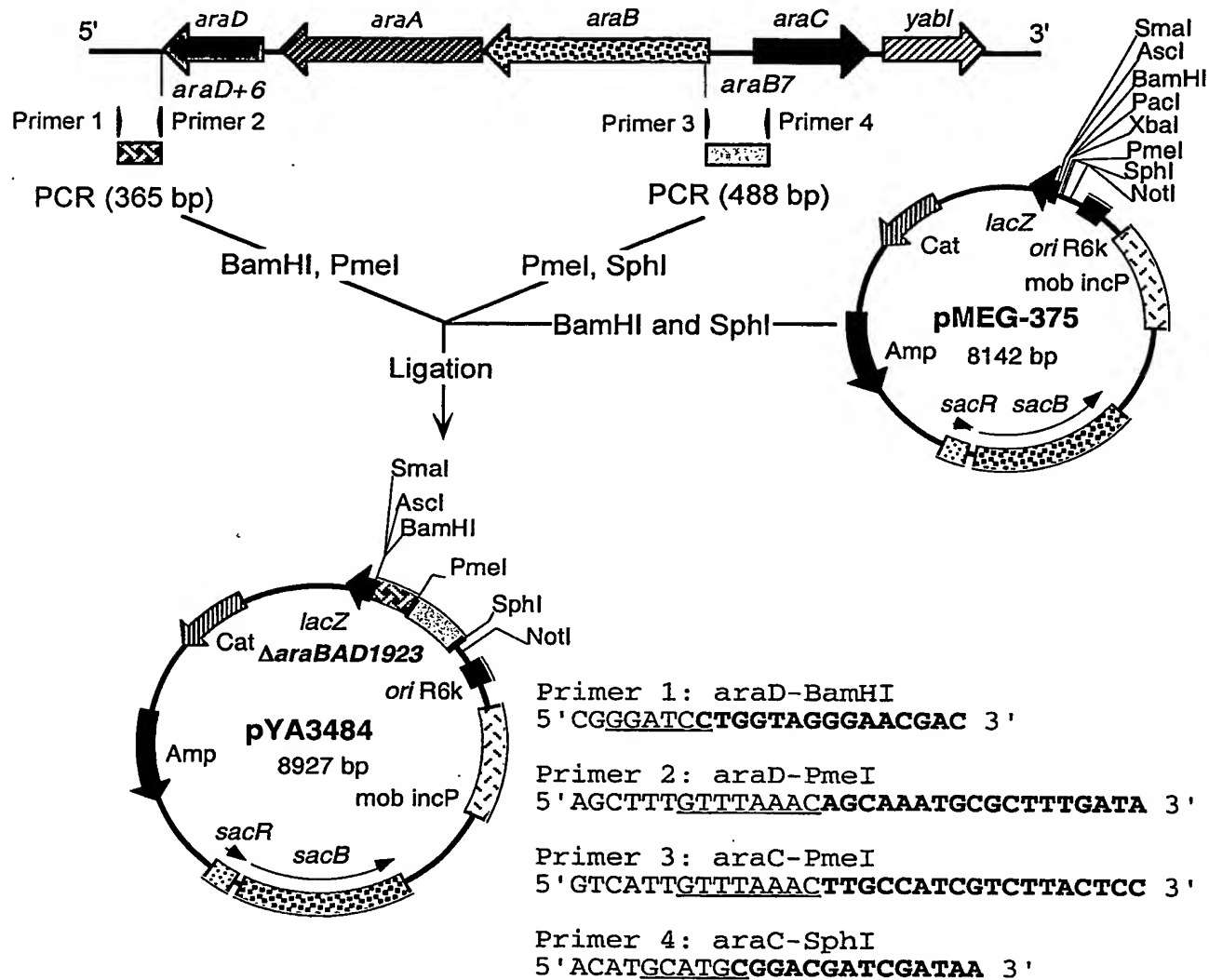


FIGURE 9. Construction of suicide vector for Δ araBAD1923



In *Salmonella* chromosome:

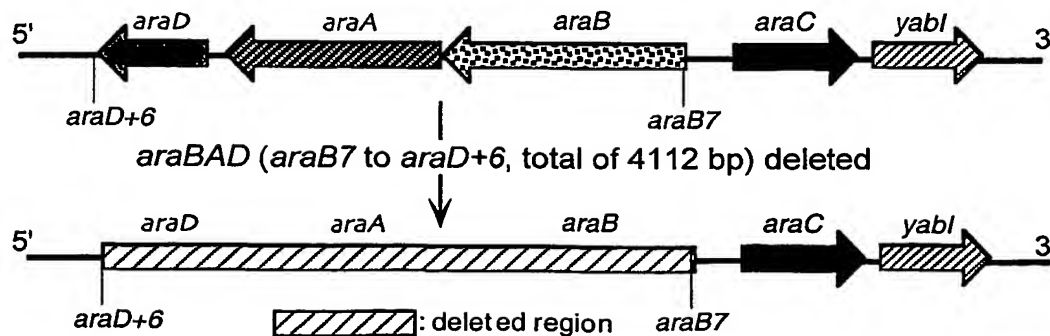
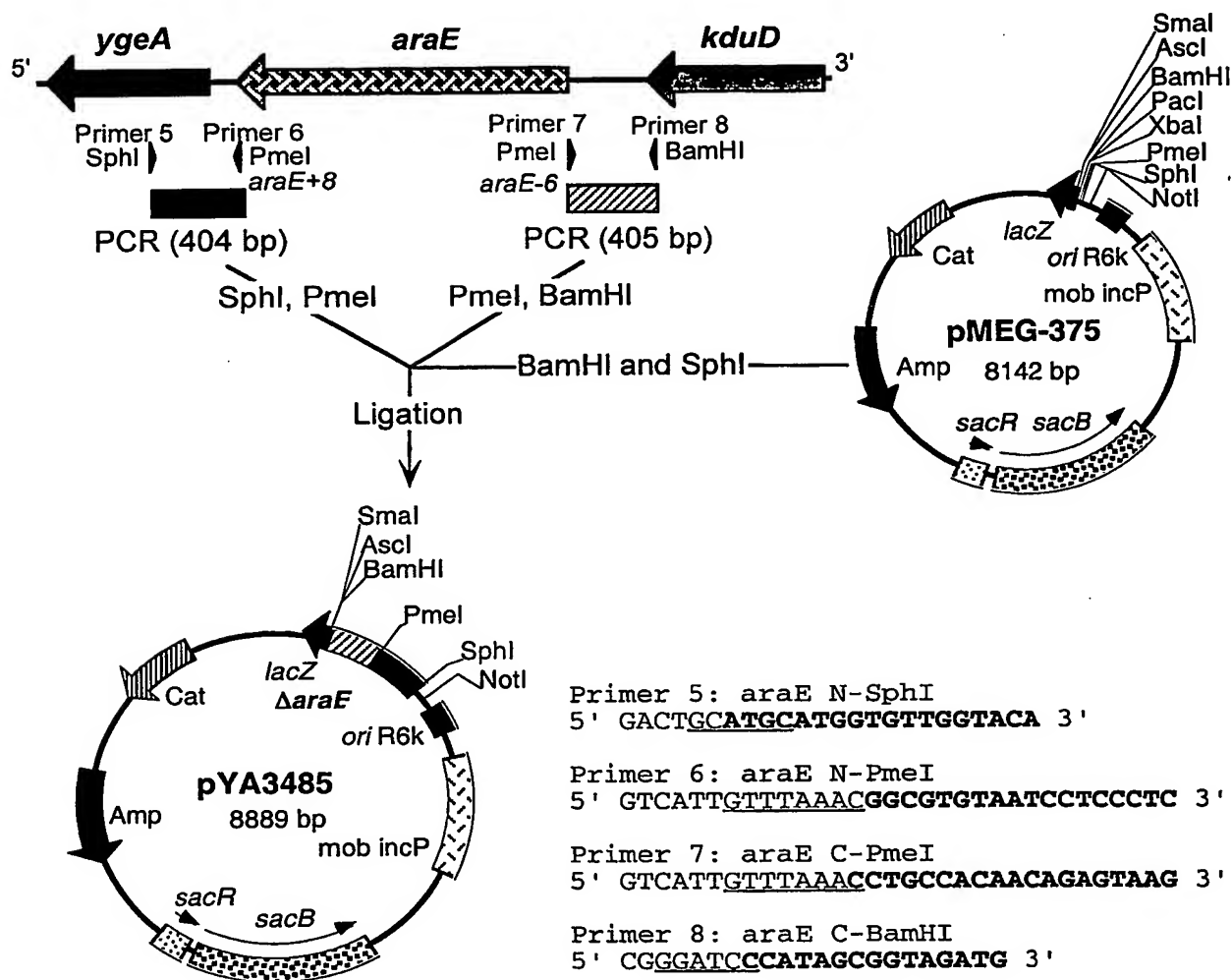
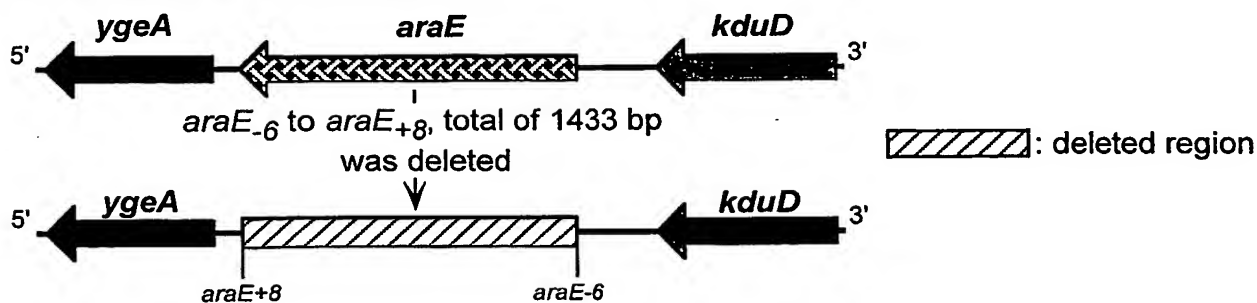
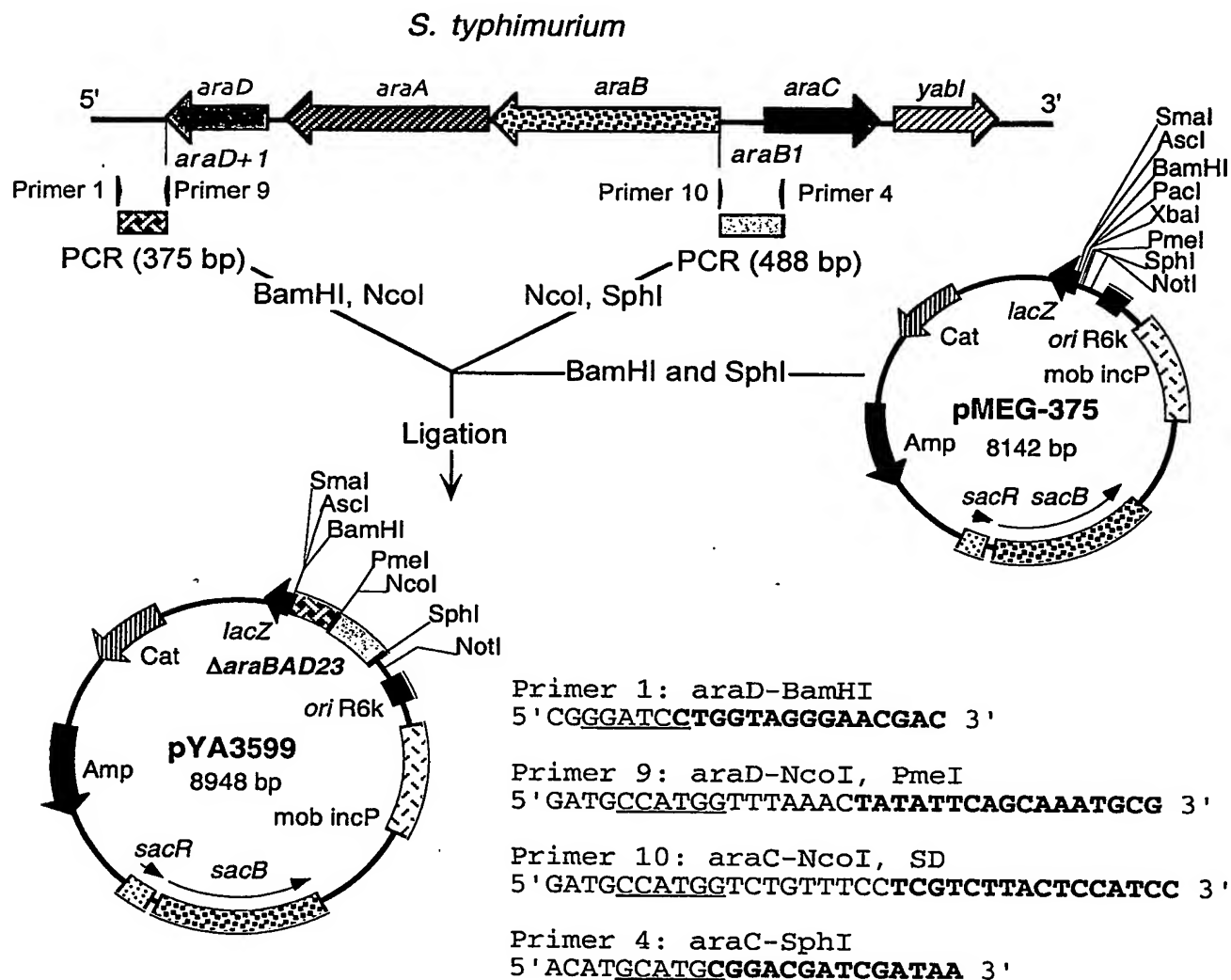
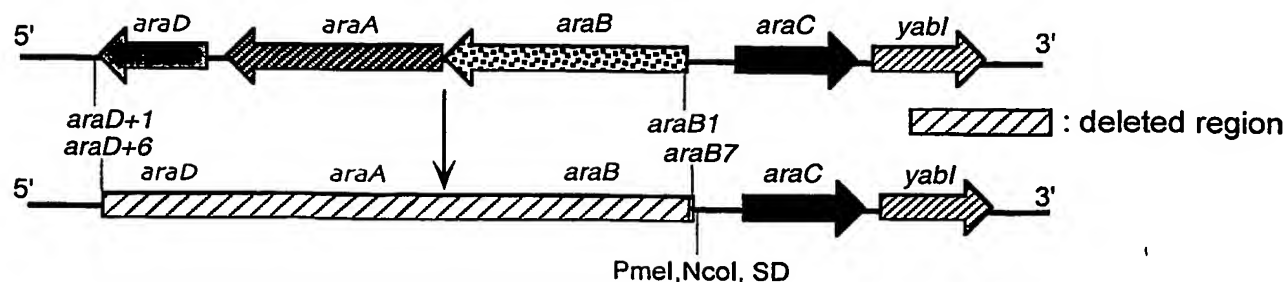


FIGURE 10. Construction of suicide vector for $\Delta araE25$ In *Salmonella* chromosome:

ygeA: Function unknown

araE: Low-affinity L-arabinose transport; L-arabinose proton symport

kduD: 2-deoxy-D-gluconate 3-dehydrogenase

FIGURE 11. Construction of suicide vector for Δ araBAD23**In *Salmonella* chromosome:**

araBAD (*araB1* to *araD+1*, total of 4111 bp) deleted and
 SD, NcoI and PmeI sites inserted

FIGURE 12. Construction of suicide vectors for $\Delta araBAD23$ $c2$ $lacI::rrfG$ TT, $\Delta araBAD23$ $c2::rrfG$ TT and $\Delta araBAD23$ $lacI::rrfG$ TT

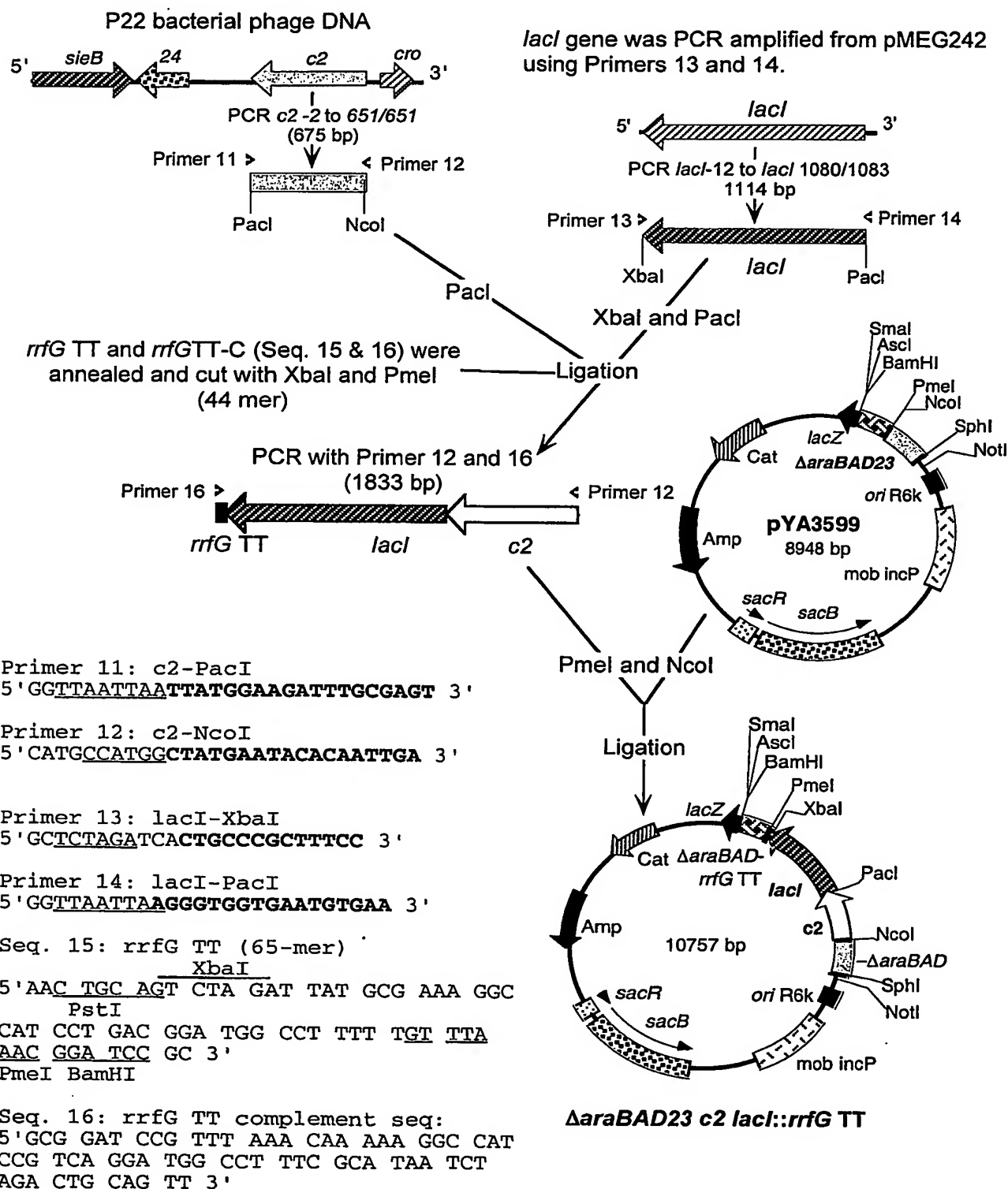


FIGURE 12. continued

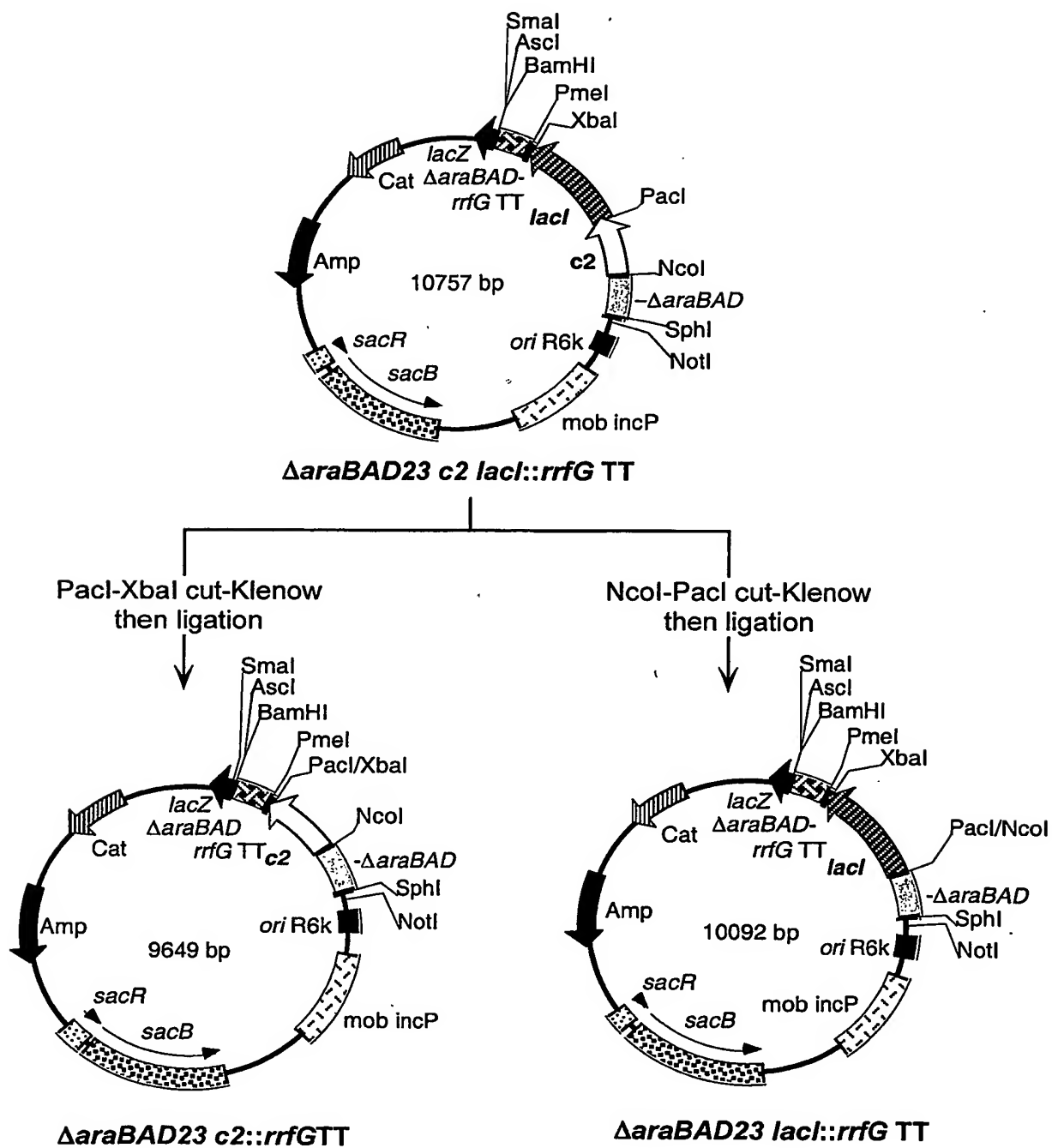


FIGURE 12. continued

In *S. typhimurium* chromosome:

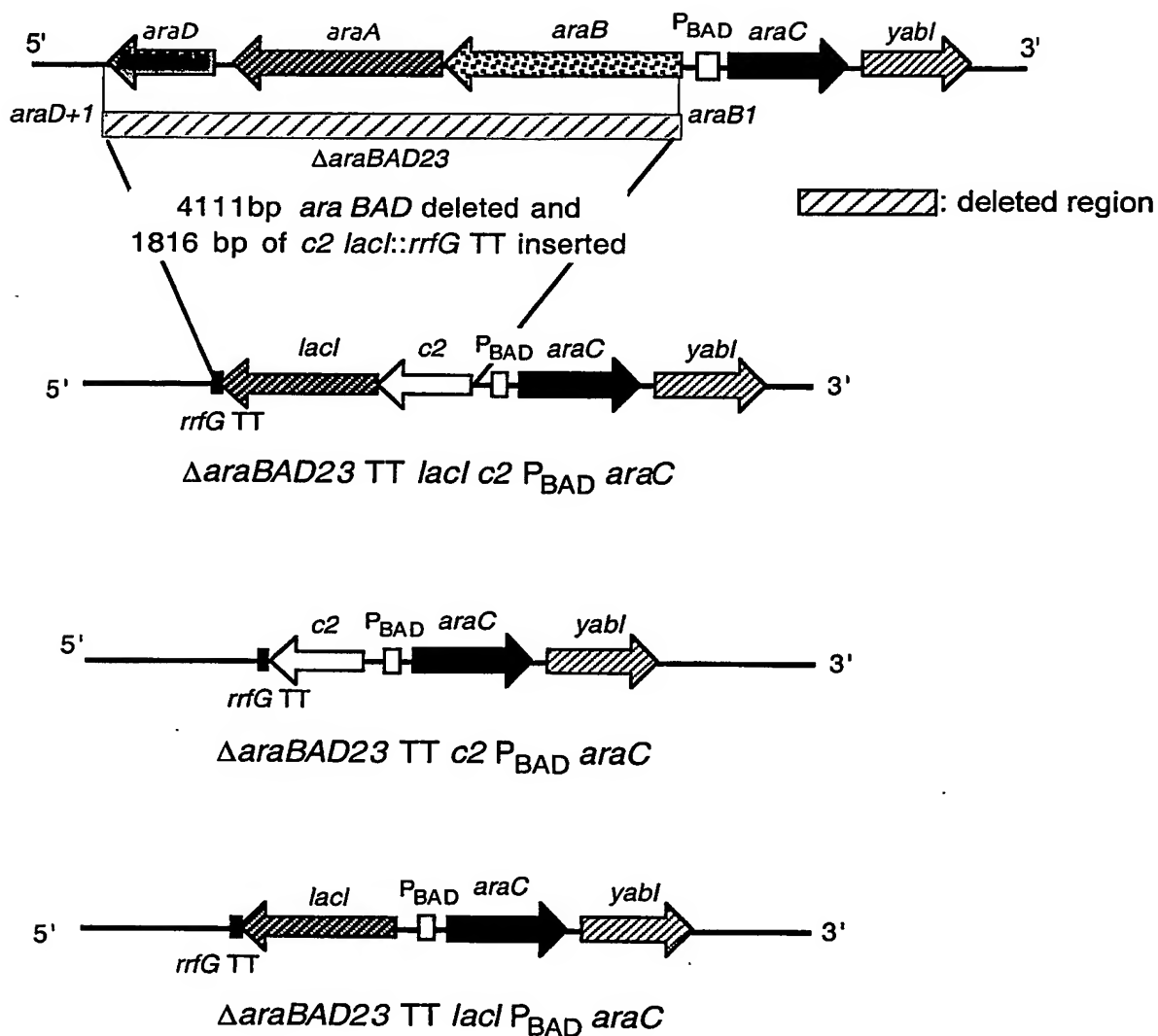


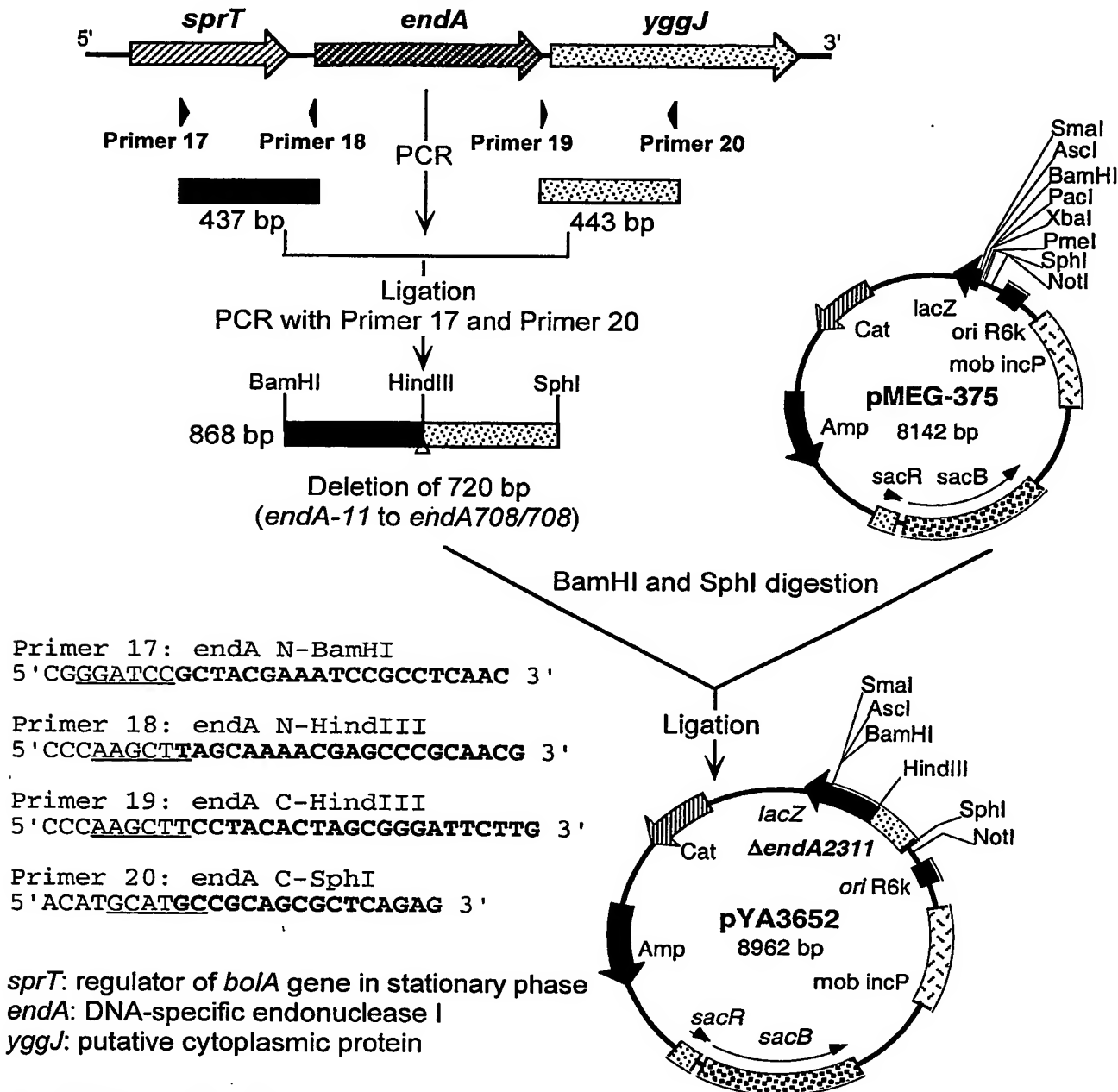
FIGURE 13. Construction of suicide vector for $\Delta endA2311$ 

FIGURE 14. Construction of suicide vector for $\Delta endA23::TT$ *araCP_{BAD} lacI* with improved *lacI* expression

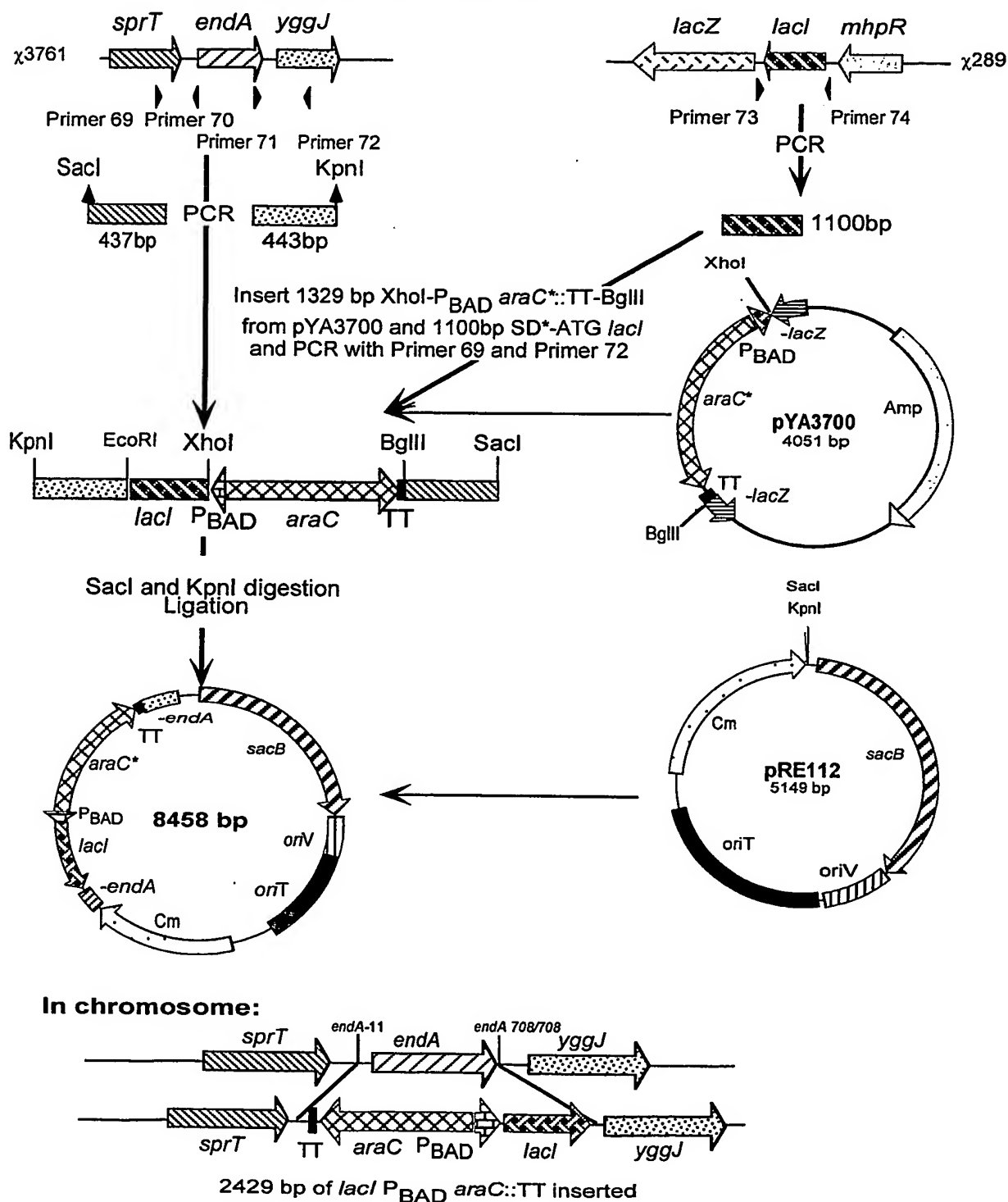
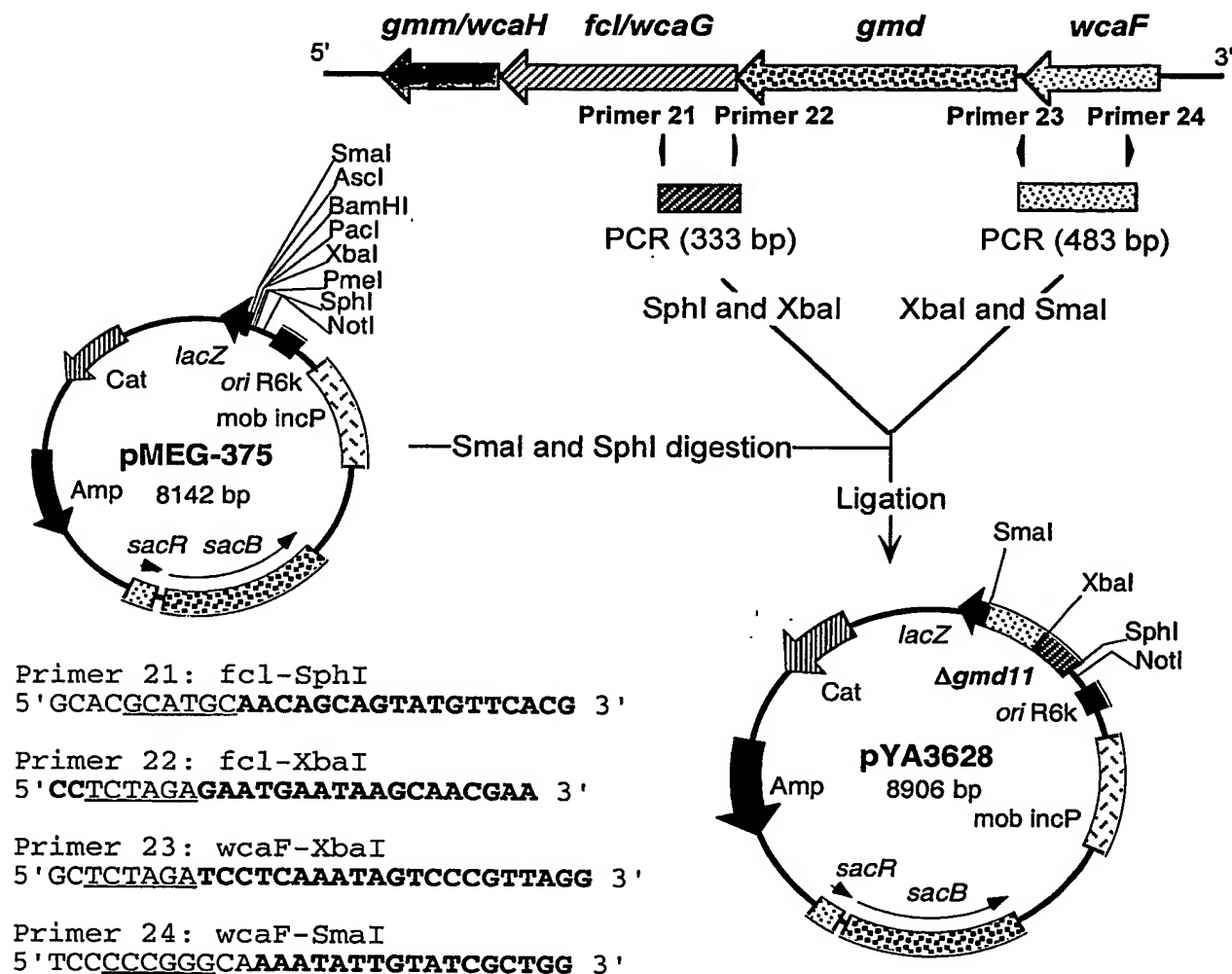


FIGURE 15. Construction of suicide vector for $\Delta gmd-11$ 

gmm/wcaH: Guanosine di-P mannose mannosyl hydrolase
fcl/wcaG: Colanic acid gene cluster, bifunctional GDP fucose synthetase
gmd: Fucose biosynthesis; GDP-D-mannose 4,6-dehydratase
wcaF: Involved in lipopolysaccharide biosynthesis, putative acyltransferase

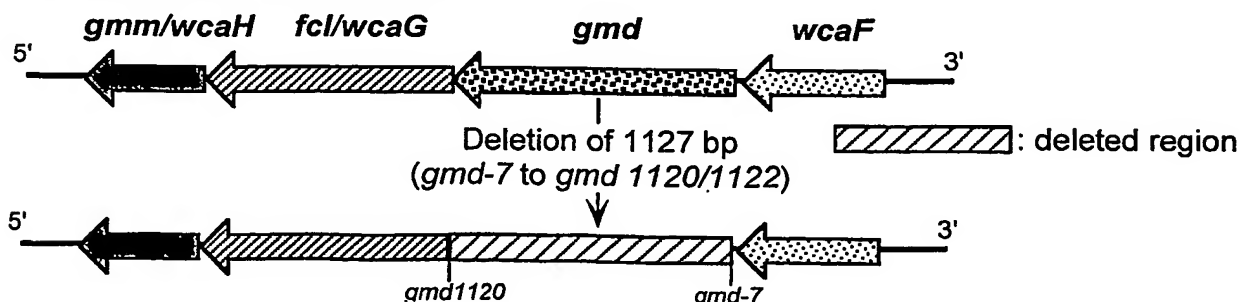
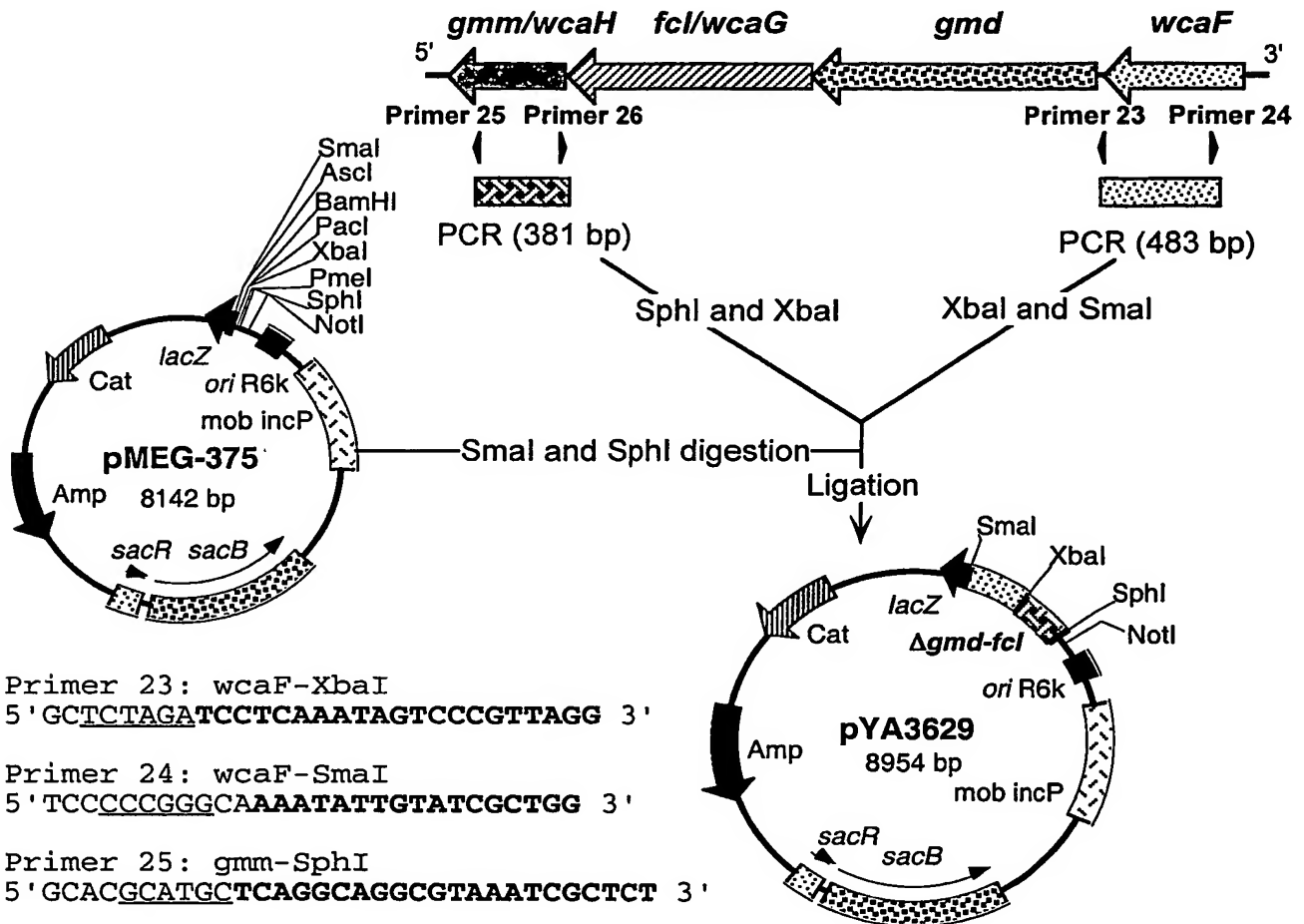
In *Salmonella* chromosome:

FIGURE 16. Construction of suicide vector for $\Delta(gmd-fcl)$ -26

gmm/wcaH: Guanosine di-P mannose mannosyl hydrolase
fcl/wcaG: Colanic acid gene cluster, bifunctional GDP fucose synthetase
gmd: Fucose biosynthesis; GDP-D-mannose 4,6-dehydratase
wcaF: Involved in lipopolysaccharide biosynthesis, putative acyltransferase

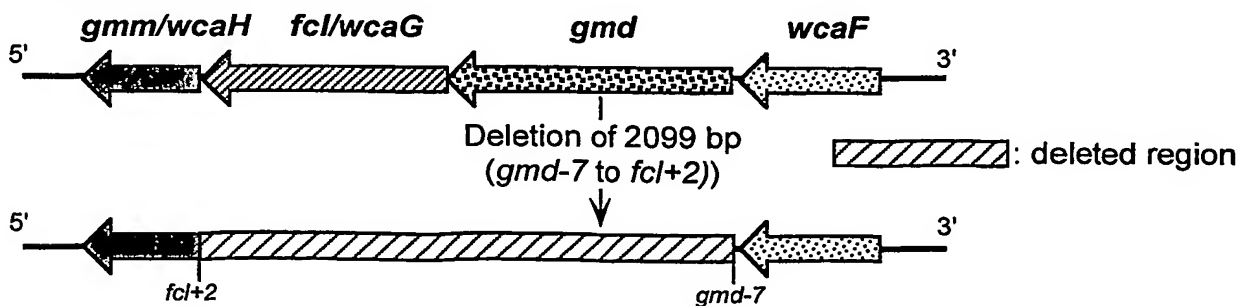
In *Salmonella* chromosome:

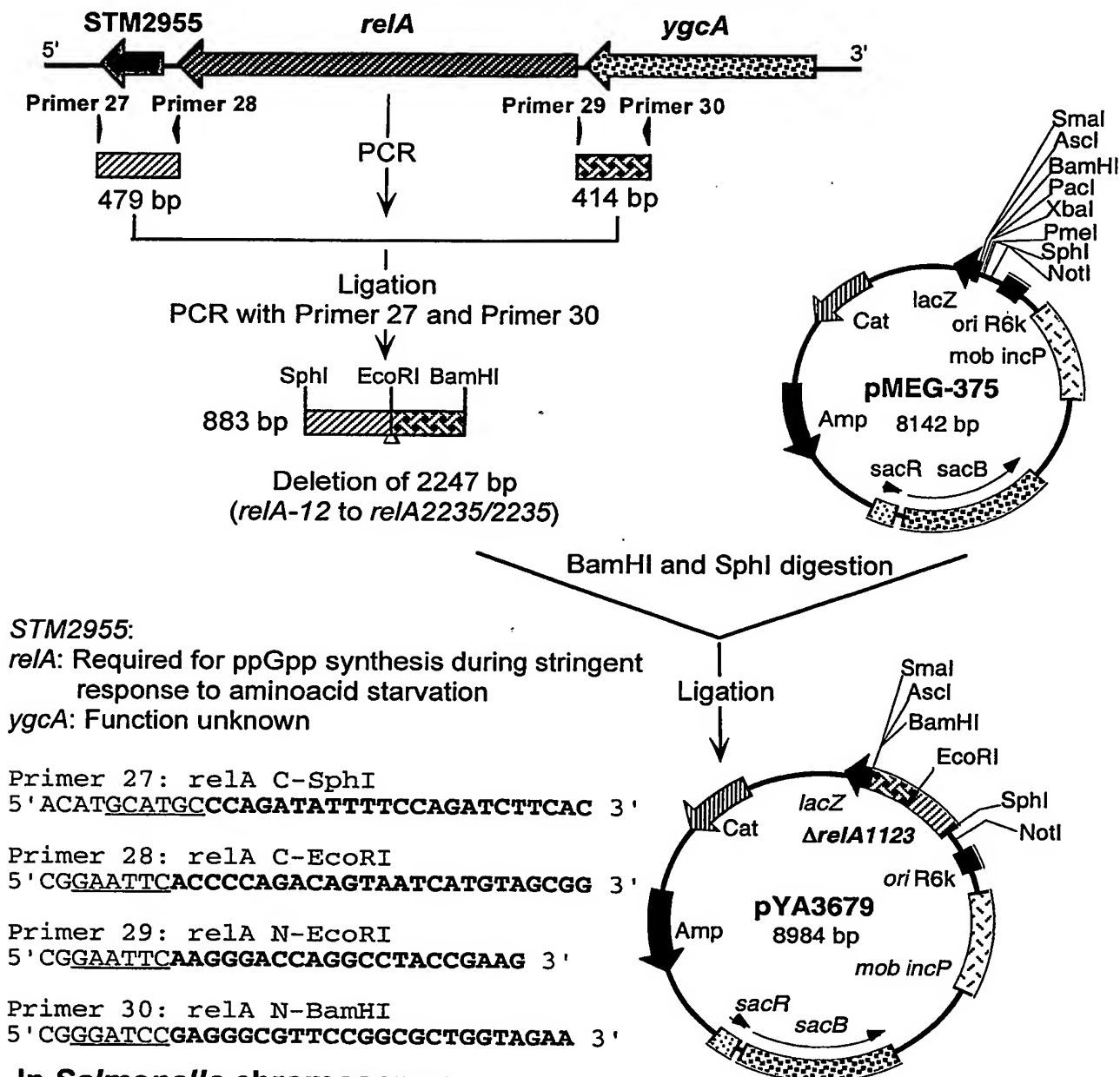
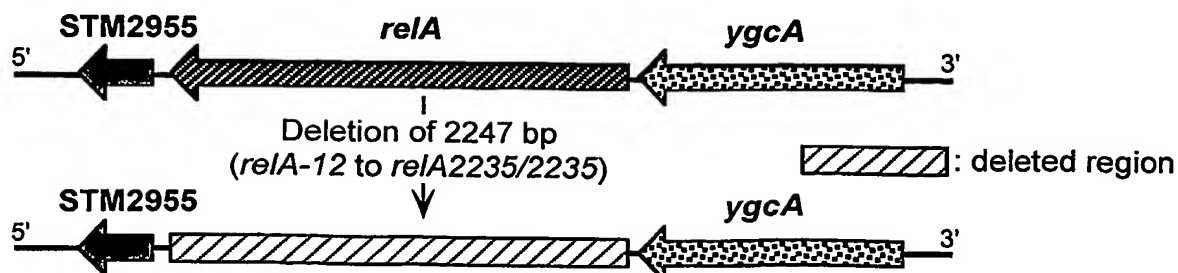
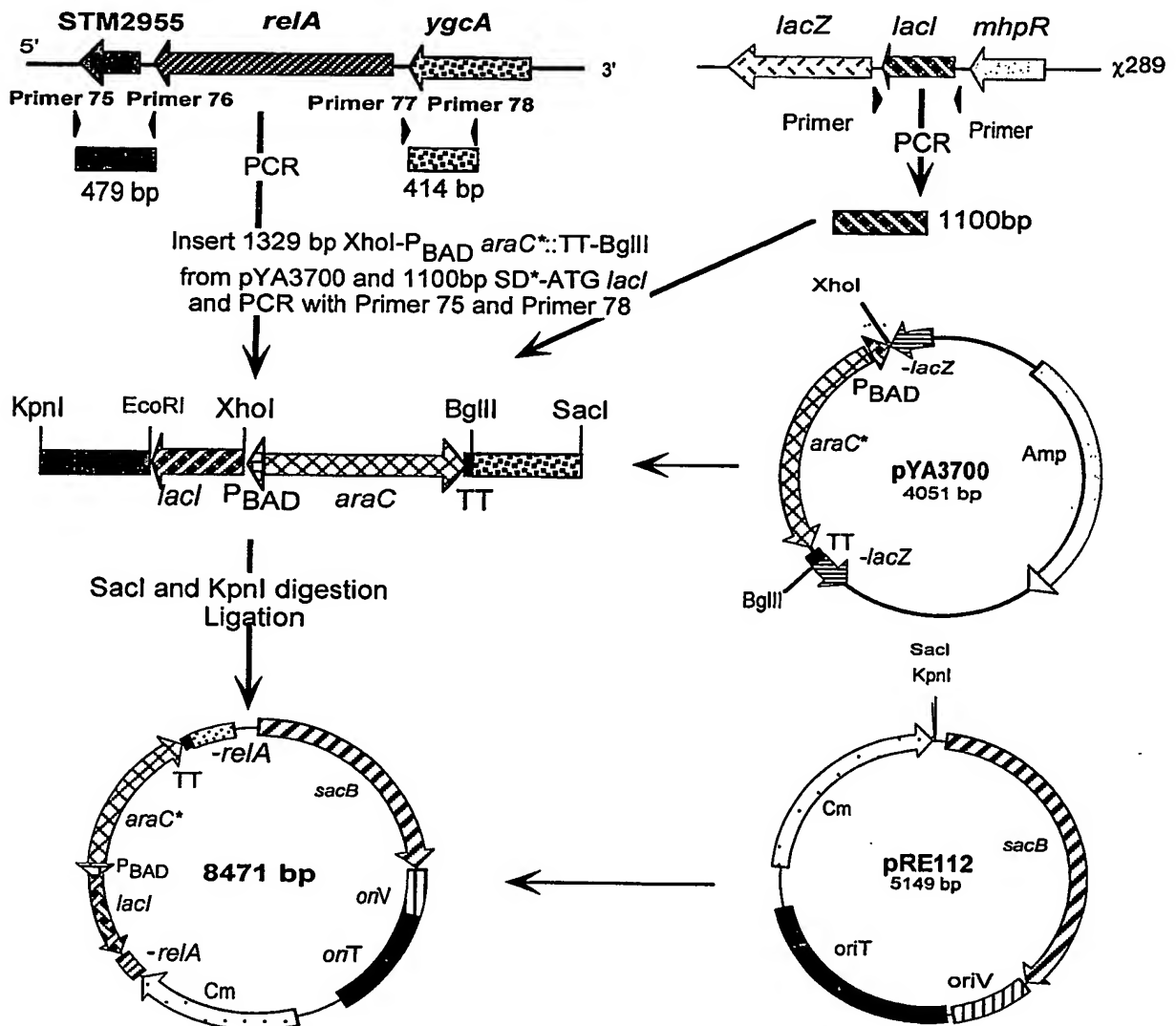
FIGURE 17. Construction of suicide vector for $\Delta relA1123$ In *Salmonella* chromosome:

FIGURE 18. Construction of suicide vector for $\Delta relA11::TT$ *araCP_{BAD} lacI* with improved *lacI* expression



In chromosome:

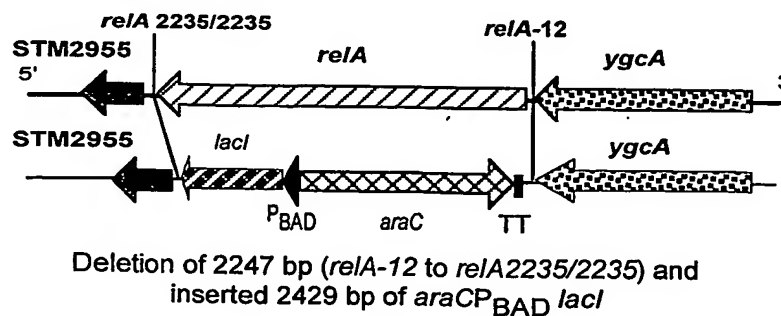
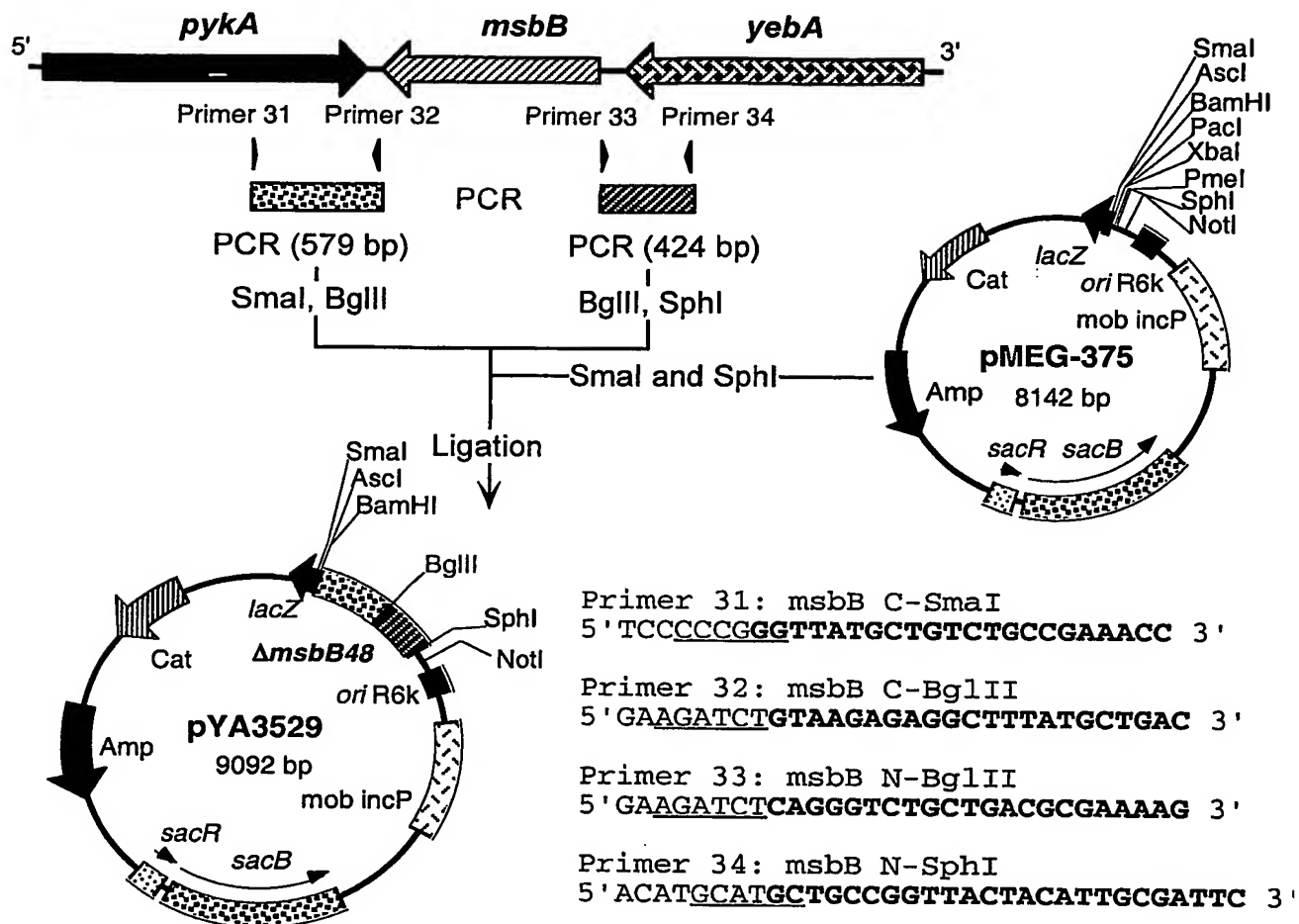


FIGURE 19. Construction of suicide vector for $\Delta msbB48$



pykA: Pyruvate kinase A (II)

msbB: Role in outer membrane structure; myristoyl transferase in lipid A biosynthesis

yebA: Putative peptidase

In *Salmonella* chromosome:

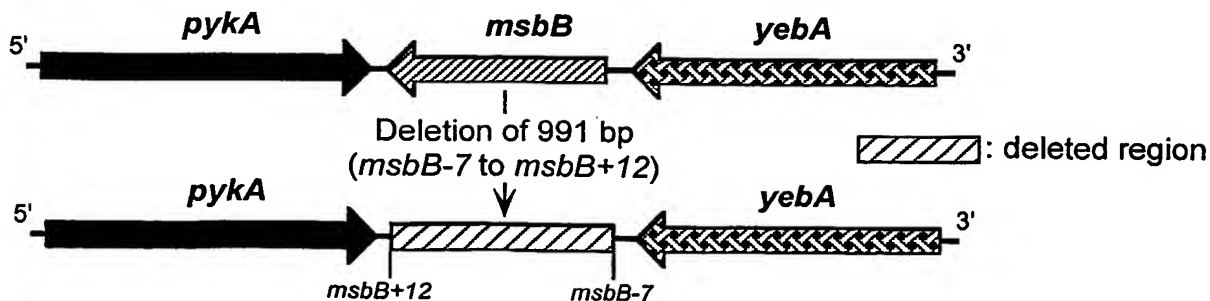


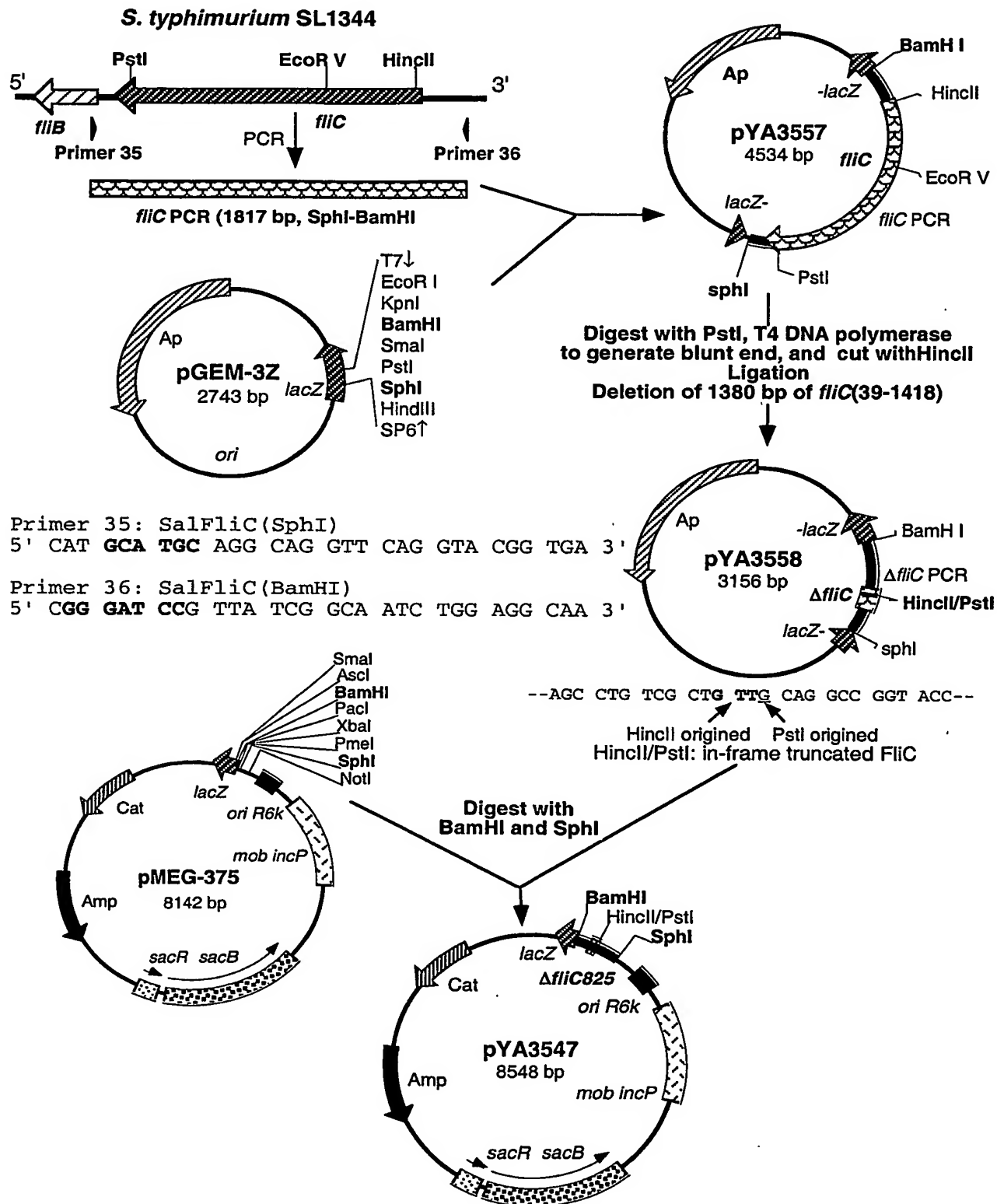
FIGURE 20. Construction of suicide vector for $\Delta fliC825$ 

FIGURE 21. Construction of suicide vector for $\Delta fljB217$

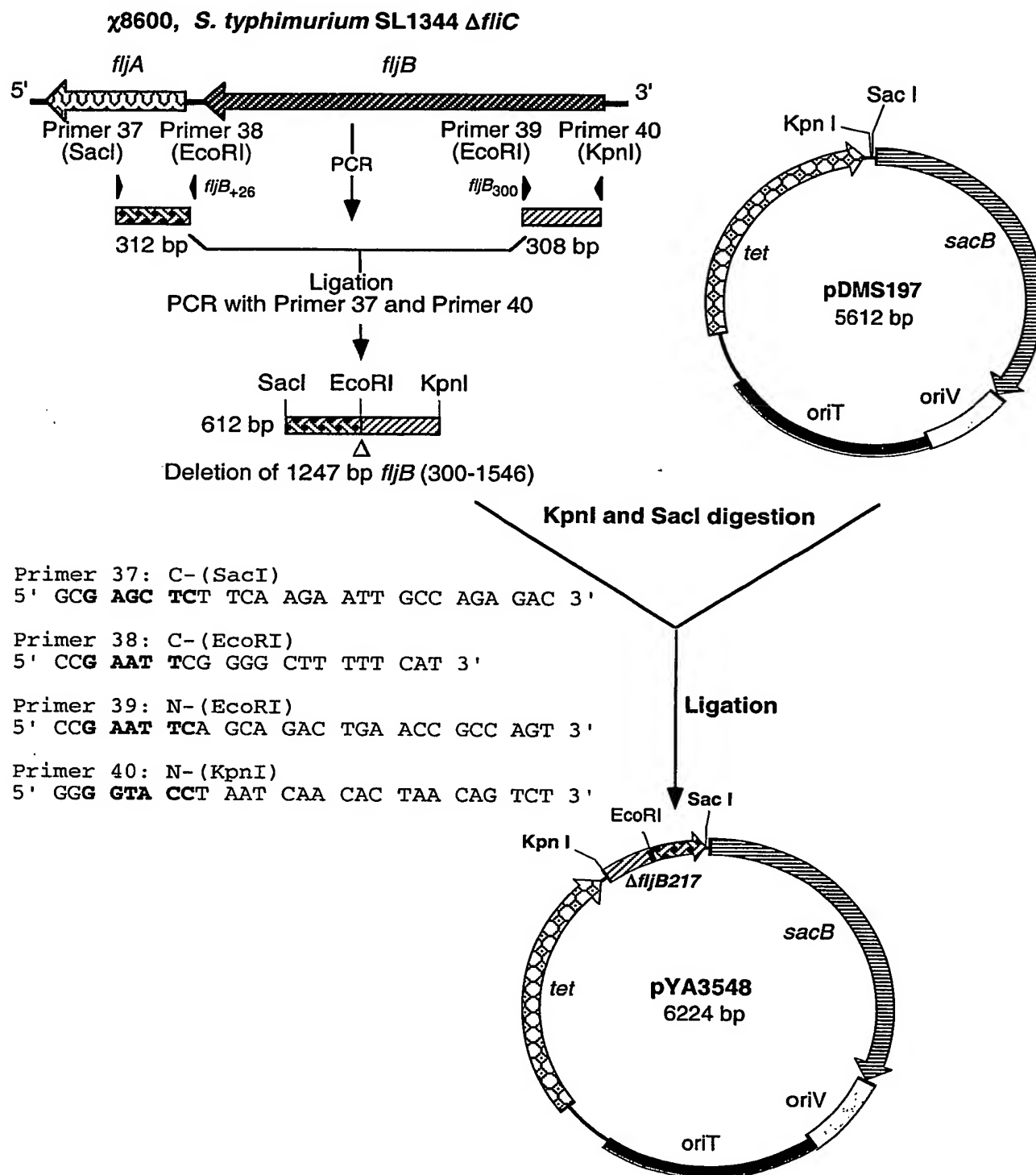
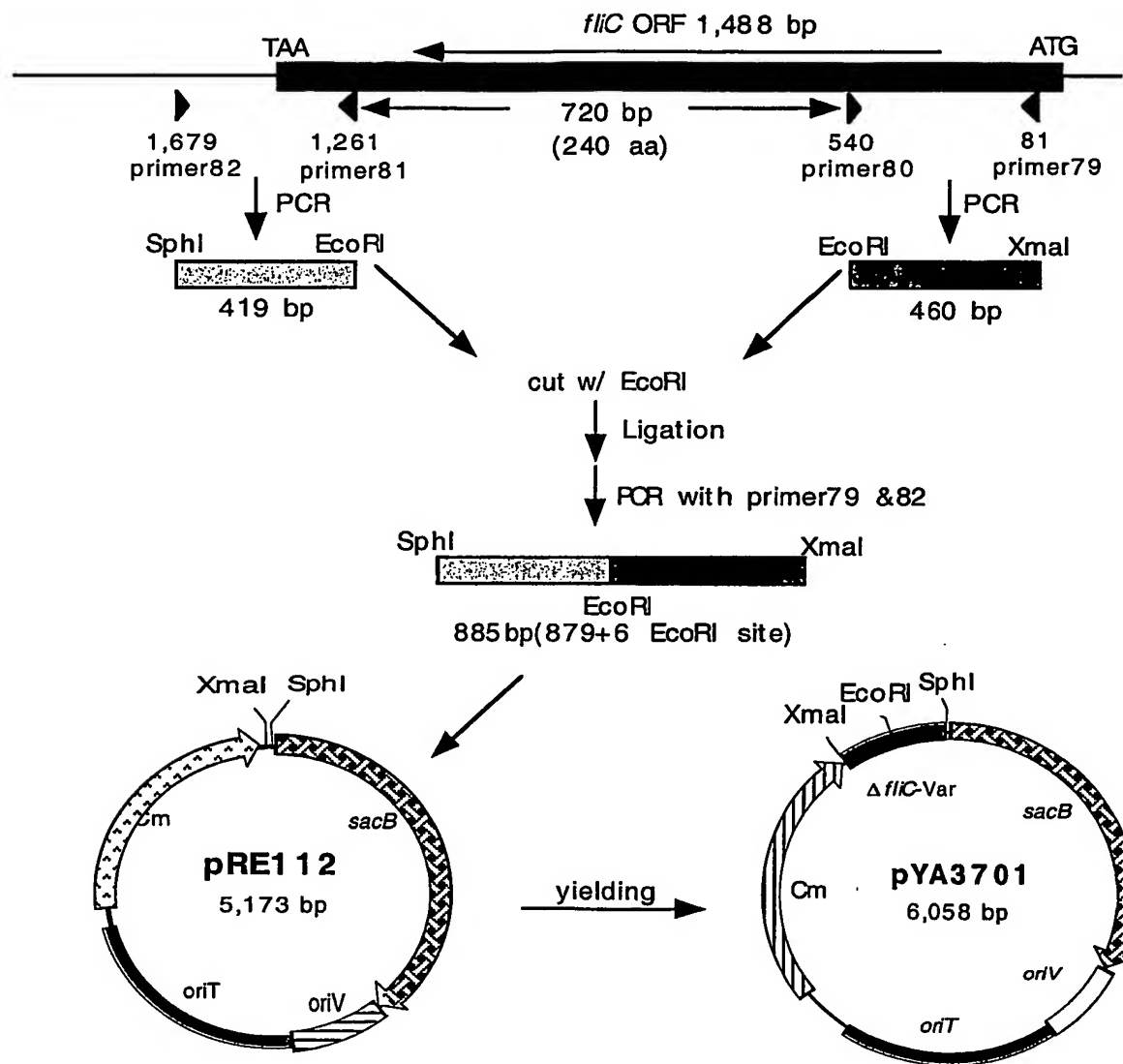


Figure 22. Construction of a suicide vector for transfer of $\Delta fliC$ -Var mutation



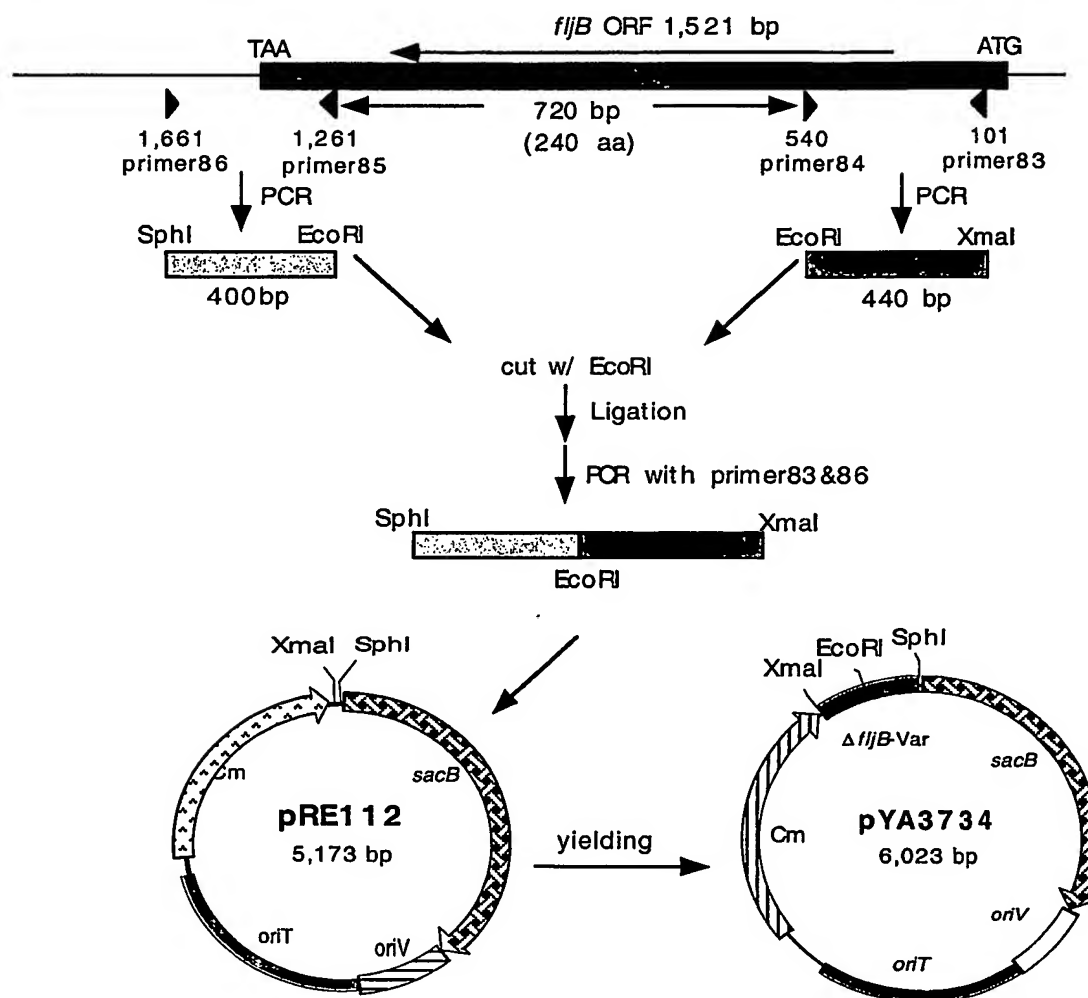
primer 79: $\Delta fliC$ 1 XmaI/bp81-104
 5'-TCCGCCGGGGCTA TGG AGCGTCTGT CTT OGG G-3'

primer 80: $\Delta fliC$ 2 EcoRI/bp540-516
 5'-GGGAAT TOCTTATAT TTT TGT TGCACATTCAG-3'

primer 81: $\Delta fliC$ 3 EcoRI/bp1261-1285
 5'-GGGAAT TCA GGT TAC GTT CTGACCTGGGTGGG-3'

primer 82: $\Delta fliC$ 4 SphI/bp1679-1655
 5'-ACA TGCATGOOGTCT TAT CCAGCG TGA TTT TCCA-3'

Figure 23. Construction of the suicide vector for the $\Delta fliB$ -Var deletion mutation



primer 83: delV.fliC 1 XmaI/bp81-104
 5'-TCCCCCGGCT GGT CTGCGT ATCAAC AGC-3'
 primer 84: delV fliC 2 EcoRI/bp540-516
 5'-GGGAAT TCA TCA TAC GCT TTCTGCAOG TT-3'
 primer 85: delV fliC 3 EcoRI/bp1261-1285
 5'-GGGAAT TCCAGA AAA TTG ATGOOG CGC TG-3'
 primer 86: delV fliC 4 SphI/bp1679-1655
 5'-ACA TGCATG CCATAG AAT AAT CCC GCG GCC-3'

FIGURE 24. Construction of the suicide vector to make the $\Delta sifA26$ (in-frame deletion) mutation

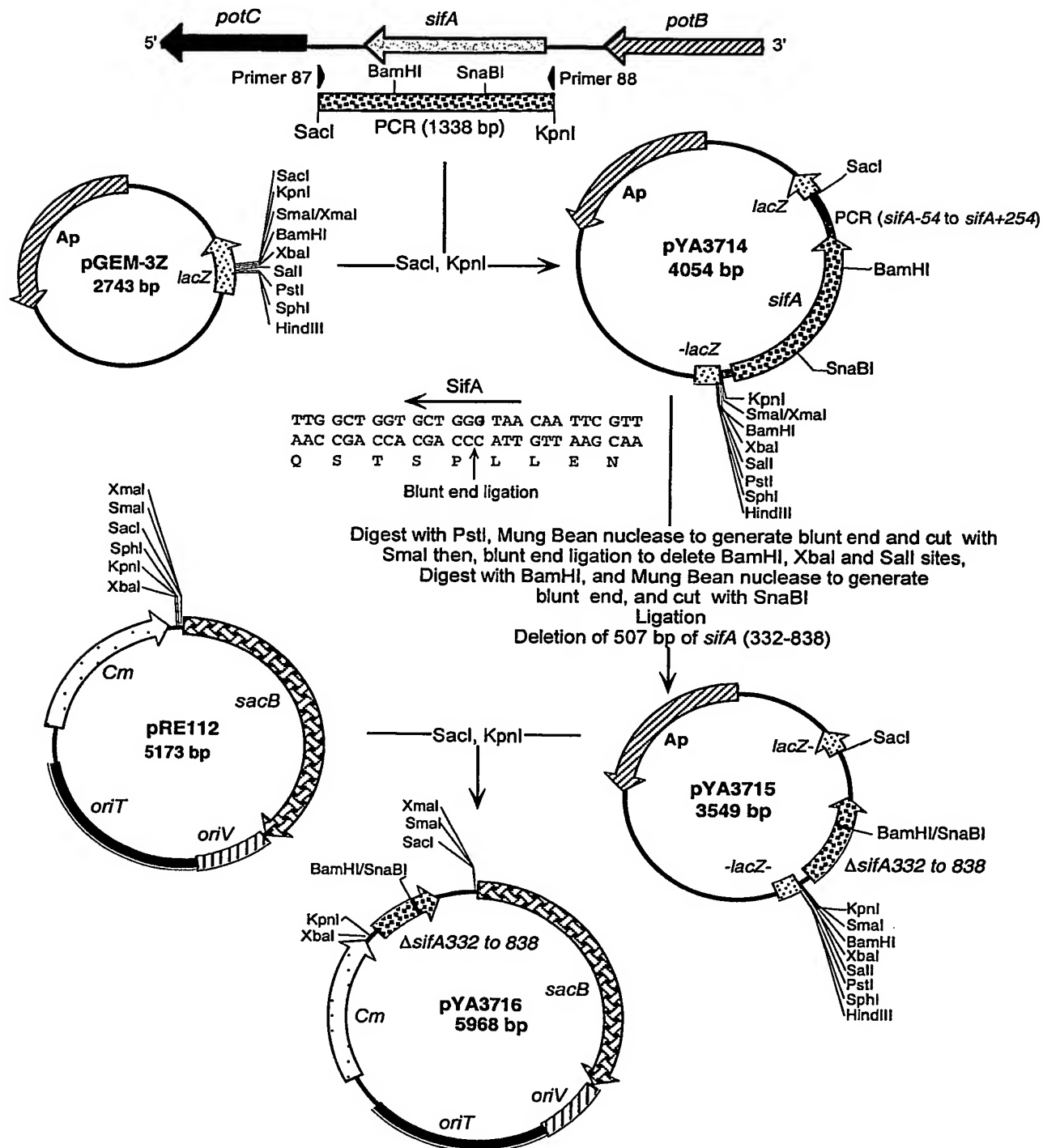
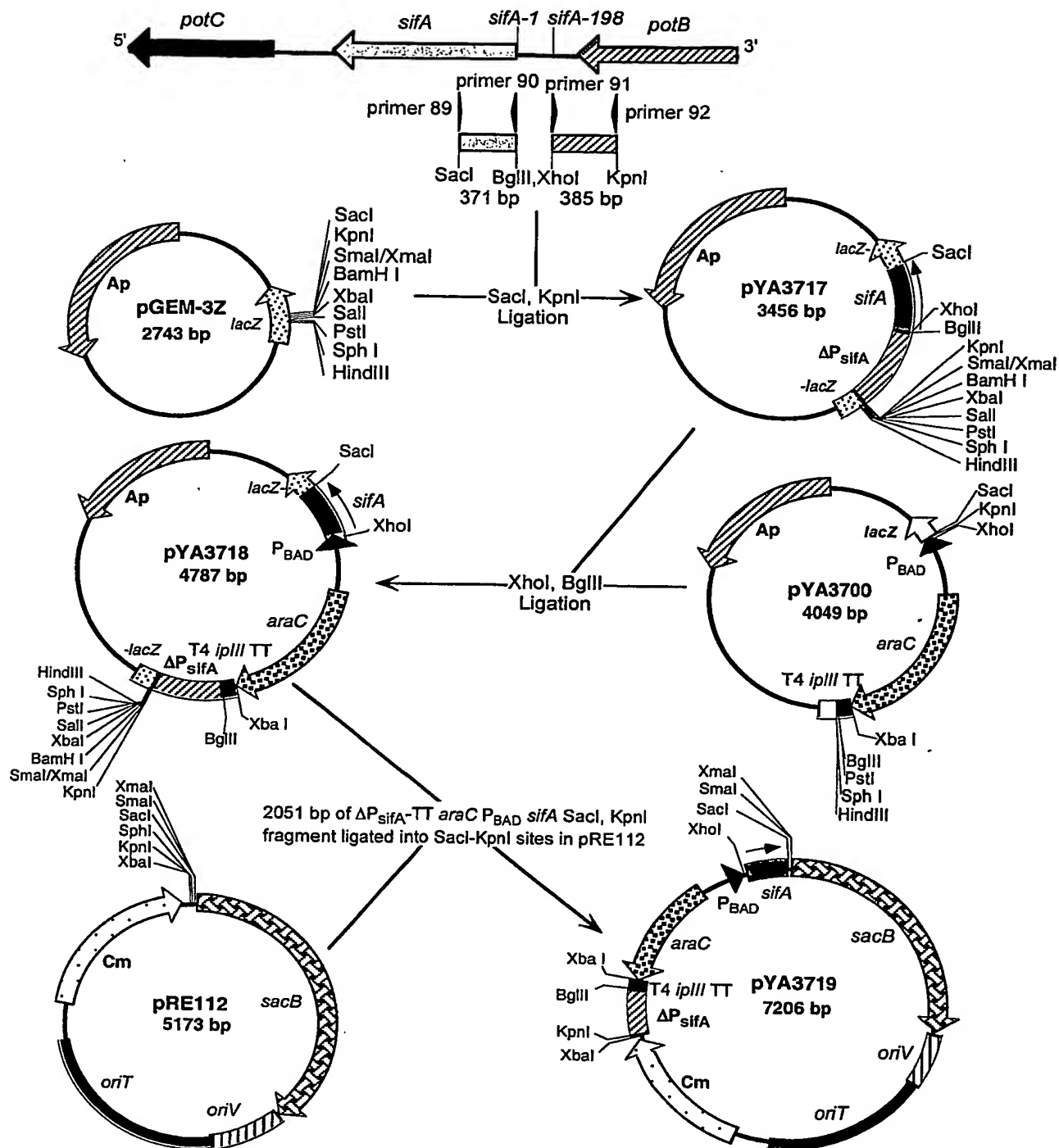


FIGURE 25. Construction of suicide vector $\Delta P_{sifA196}::TT\ araC P_{BAD} sifA$

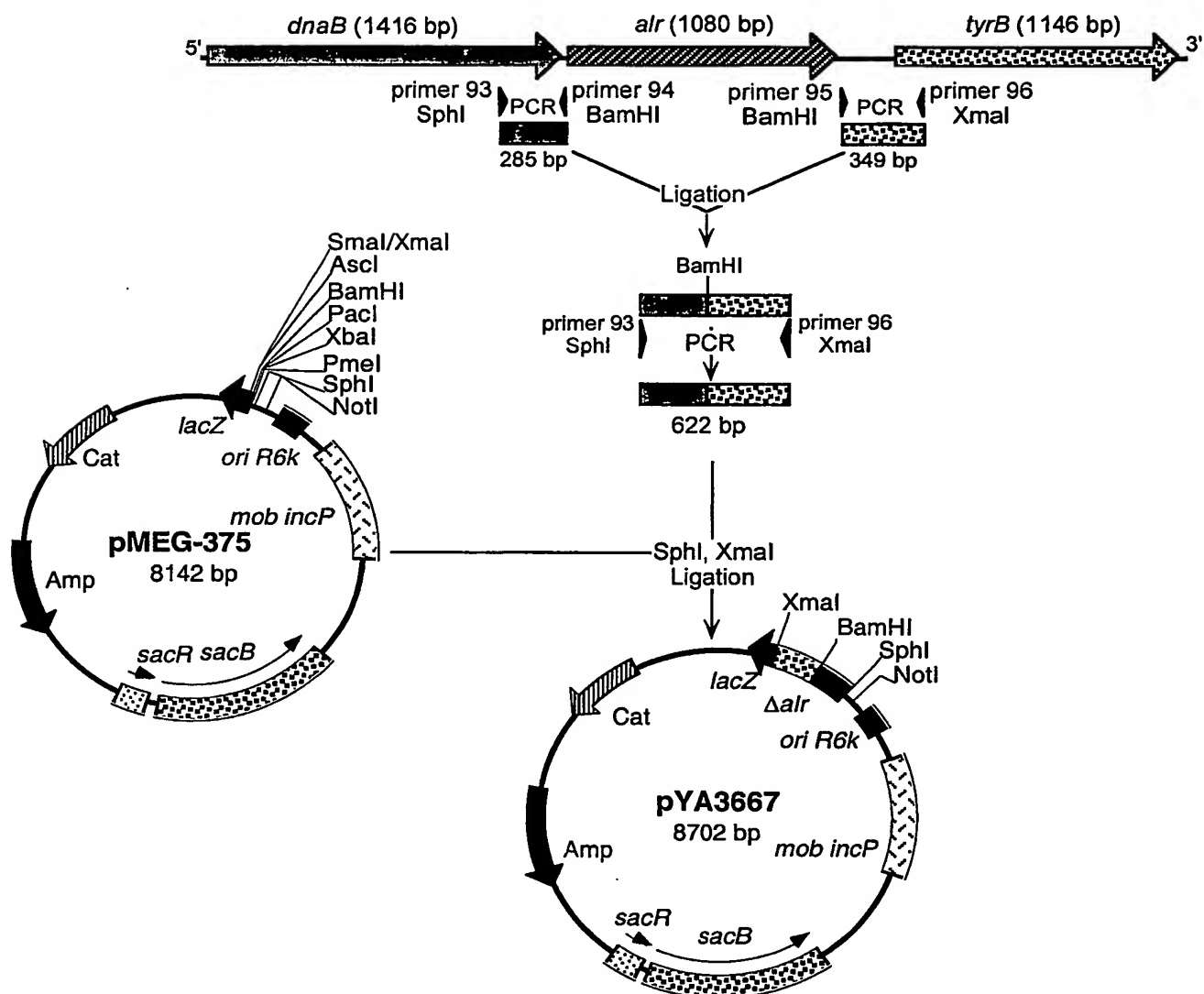


Primer 89: GCAAGAGCTCCTCTTCGTTTTGATCCATG (*sifA*-SacI)

Primer 90: GCCGGATCCAGATCTTATCTACTCGAGAGGAAAAACGCTATGCCGATTACTATAGGG (*sifA*-XhoI BglII)

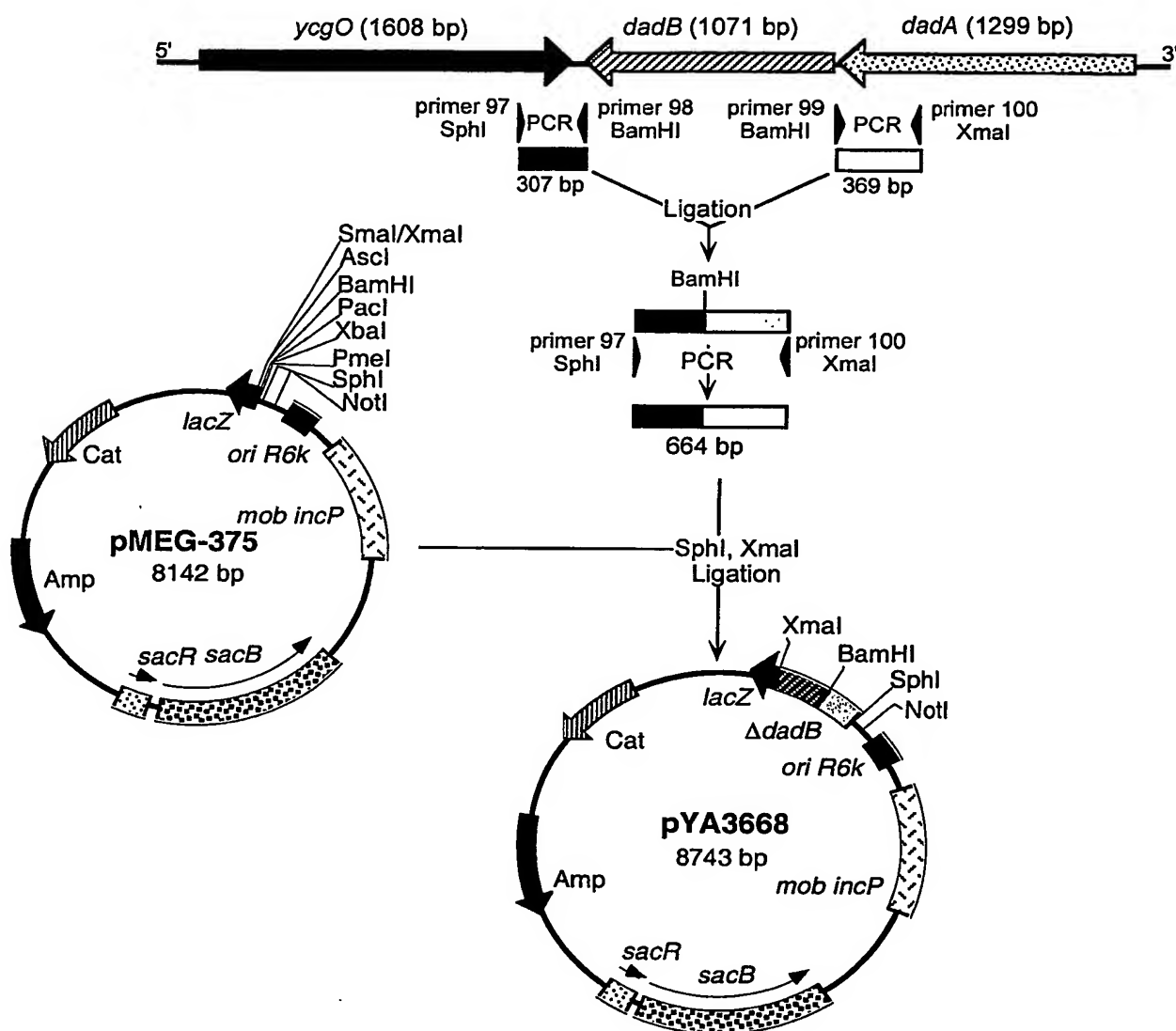
Primer 91: CCTCTCGAGTAGATAAGATCTGGATCCGGCGGATGATGTTGTAGATTG (*sifA*-XhoI BglII)

Primer 92: GCAGGTACCCGGCAATGGGCCTGTTCTAC (*sifA*-KpnI)

FIGURE 26. Construction of suicide vector with $\Delta alr-3$ mutation

Primer 93: *dnaB*-SphI
 ACATGCATGCCGCGCGGATAAACGTCCGGTGAAC
 Primer 94: *dnaB*-BamHI
 CGCGGATCCTGTATAAAGAATGACGGAGAGTTAC
 Primer 95: *tyrB*-BamHI
 CGTGGATCCGTGGCGCTTGCGCTTATCCGGCTTG
 Primer 96: *tyrB*-XmaI
 TCCCCGGGCTTCGGCTTCGGCCACCGTTTT

FIGURE 27. Construction of suicide vector with $\Delta dadB4$ mutation



Primer 97: *ycgO*-SphI
 ACATGCATGCGAATGCGAAATTCGCCGACGTG
 Primer 98: *ycgO*-BamHI
 CGCGGATCCTAATTCAGGCTAAGGCGTCGACC
 Primer 99: *dadA*-BamHI
 CGCGGATCCTTATCAGTTATGCGCGCTATGCAA
 Primer 100: *dadA*-SmaI
 TCCCCGGGCTTTAATACCGACTTACTGCAACC

FIGURE 28. Construction of suicide vector with improved $\Delta P_{mur::TT}$ *araC* P_{BAD} *murA* deletion-insertion mutation

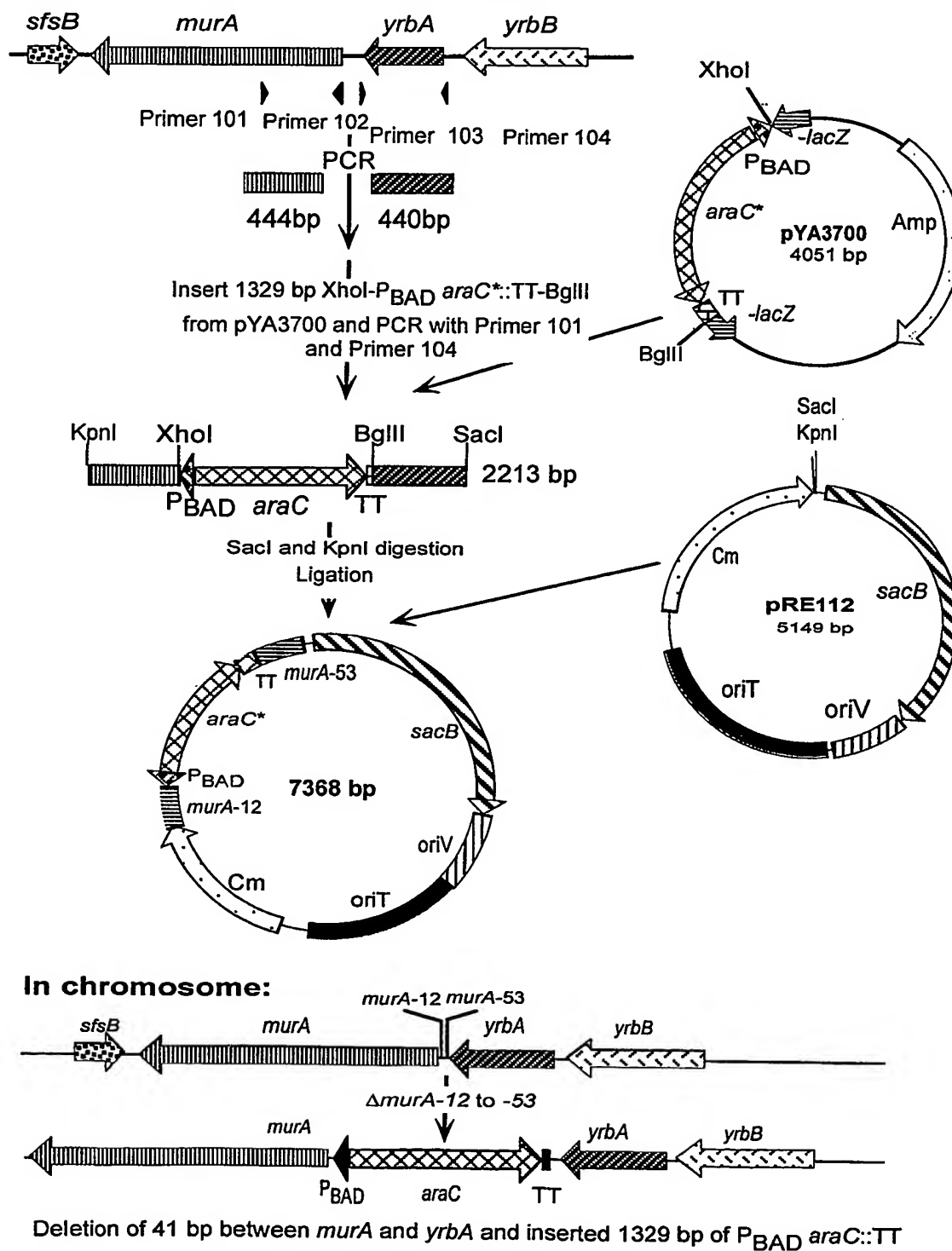
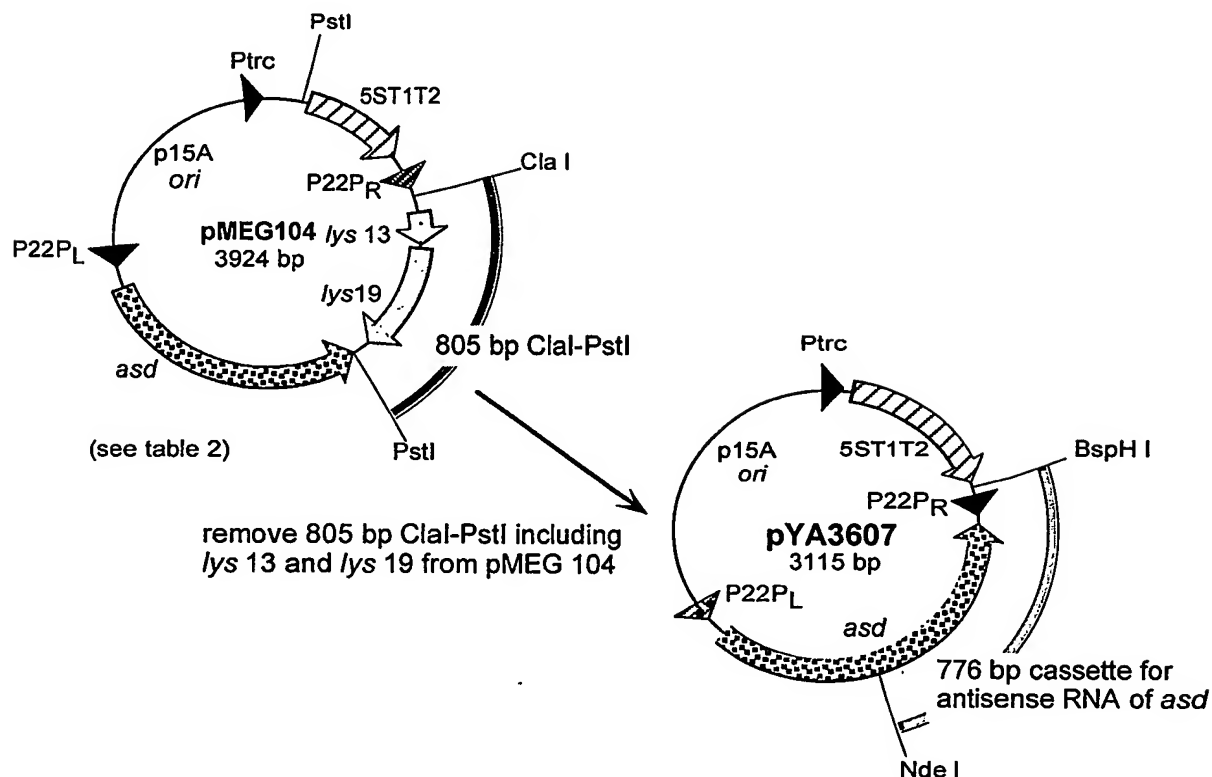


FIGURE 29. Construction of pYA3607

Sequence of antisense RNA of *asd* from P22P_R in pYA3607:

TCATGAGACA	ATAACCCTGA	TAAATGCTTC	AATAATGGAA	GATCCTACGC	TCACCCATCA
BspHI					
ATTGTGTATT	CATAGTTAAG	TCATCTTAAA	TAACTTGAC	TAAAGATTCC	TTTAGTAGAT
P22c2		OR3	OR2		
AATTTAAGTG	TTCTTTAAT	TCGGAGCGAG	TCTATGTACA	AGTCGACGGT	ATCGTGCAGC
OR1		P22cro			
TAGACTACGC	CAACTGGCGC	AGCATTCGAC	GCAGCGGCTC	GGCGGCGCCC	CATAACAAC
asd stop codon					
GGTCGCCTAC	GGTAAACGCC	GACAAGAACT	CTGGCCCCAT	GTTCAGCTTA	CGCAGACGAC
asd					
CAACCGGCGT	AGTCAACGTG	CCGGTCACCG	CCGCCGGGGT	TAATTCGCGC	ATAGTGATAT
CACGATCGTT	CGGCACCACT	TTCGCCACG	GATTATGTGC	CGCCAGCAGT	TCTTCCACCG
TCGGAATGGA	TACCTCTTTT	TTCAGCTTGA	TGGTGAACGC	CTGGCTGTGA	CAGCGCAGCG
CGCCGACGCG	CACACACAAA	CCATCAACCG	GAATCACAGA	GGCAGTATTG	AGAATCTTGT
TGGTTTCCGC	CTGGCCTTTC	CACCTCTTCG	GGCTCTGGCC	GTTATCGAGC	TGTTTGTCGA
TCCAGGGGAT	CAGGCTTCCC	GCCAGCGGTA	CGCCAAAGTT	ATCAACCGGC	AGCTCGCCGC
TGCGGGTCAA	TGCCGTAAC	TTGCGTTCAA	TATCAAGAA	TGCGGAAGAC	GGCGTCGCCA
GTTTCATCGGC	GACATGGCCA	TACAACTGAC	CCATCTGGGT	TAACAGCTCG	CGCATATG
asd					NdeI

FIGURE 30. Construction of regulatable lysis system vector pYA3646:

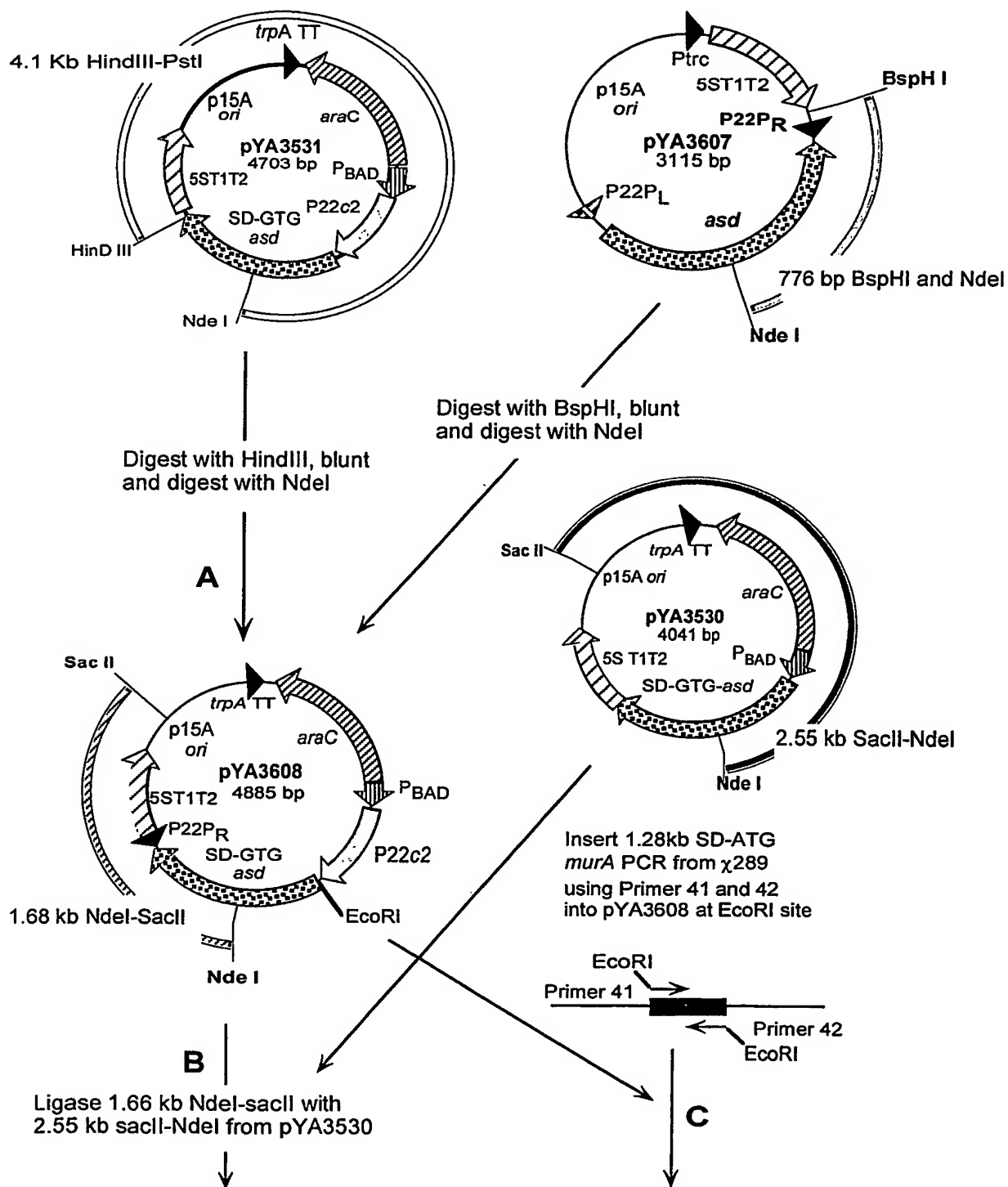


FIGURE 30. (continued)

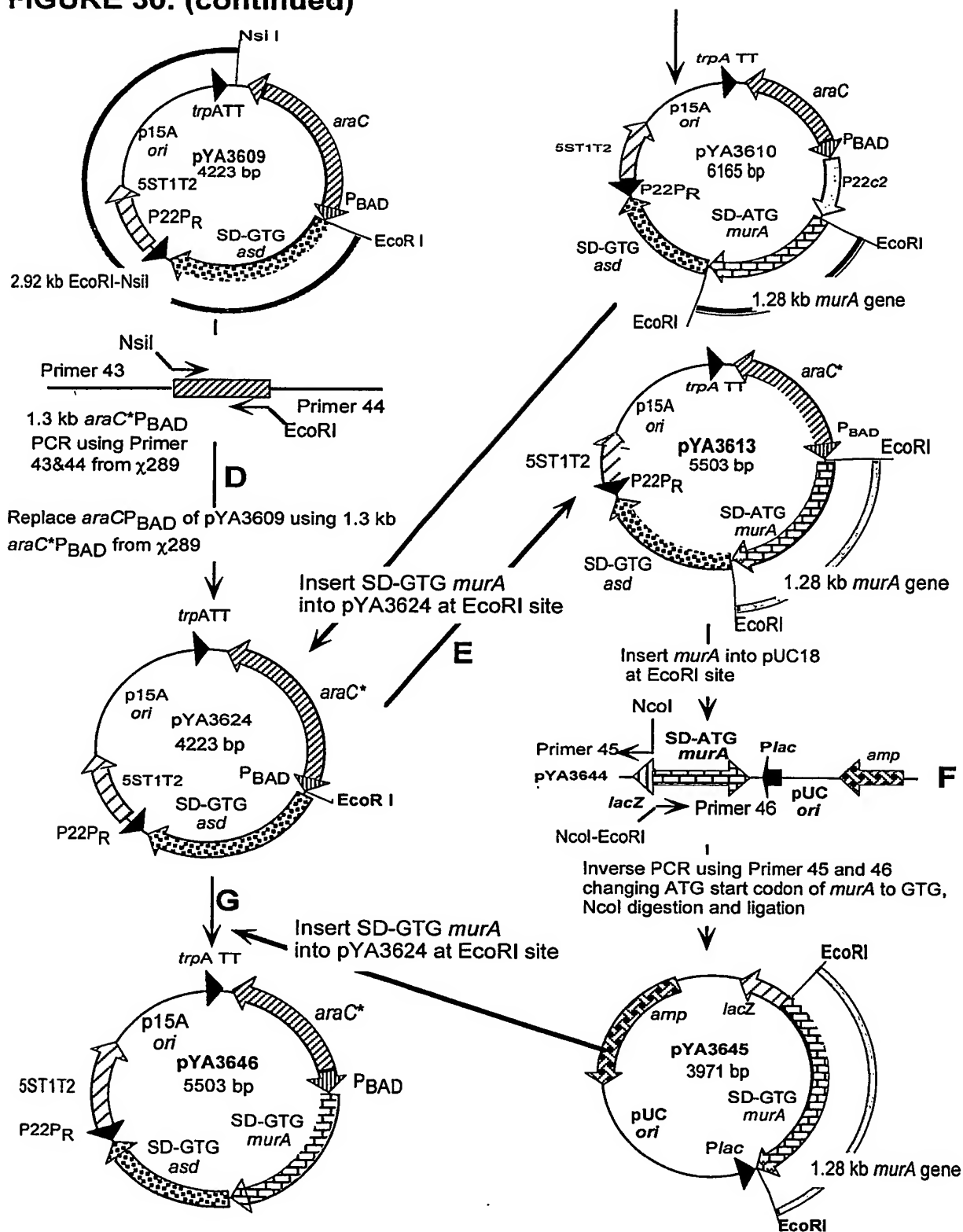
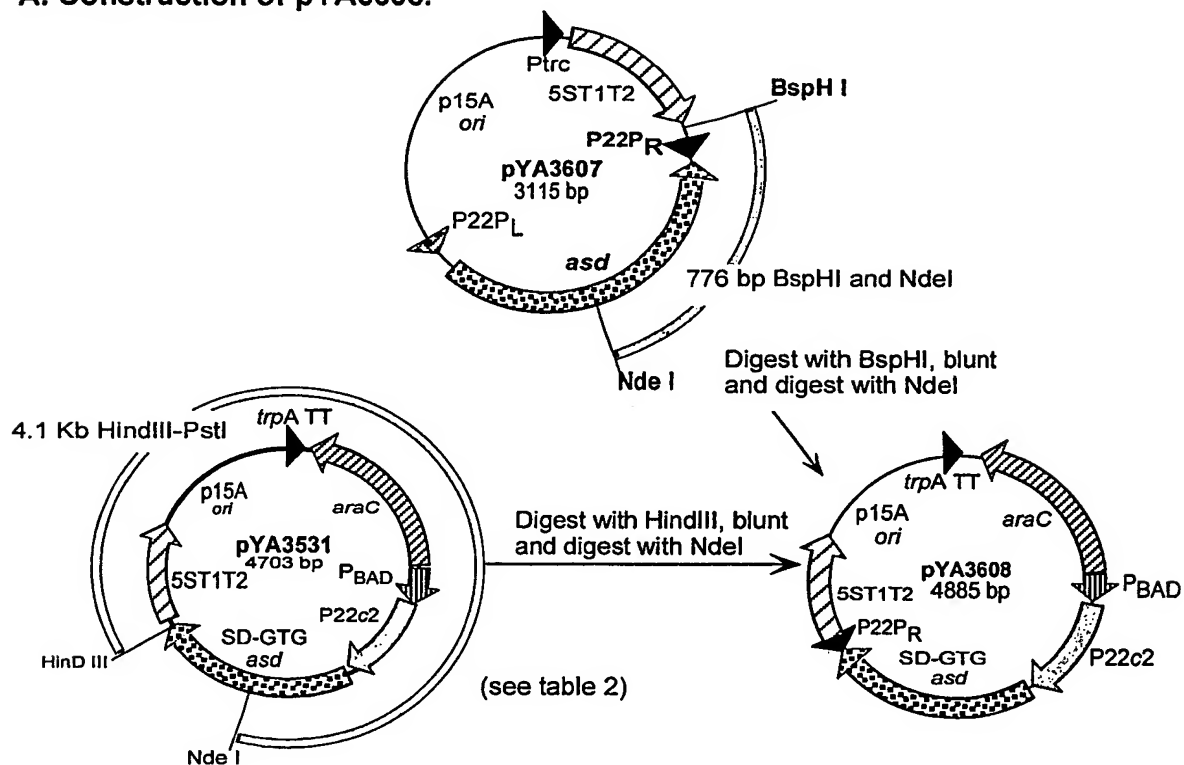


FIGURE 31. Steps in the construction of pYA3646

A. Construction of pYA3608.



B. Construction of pYA3609.

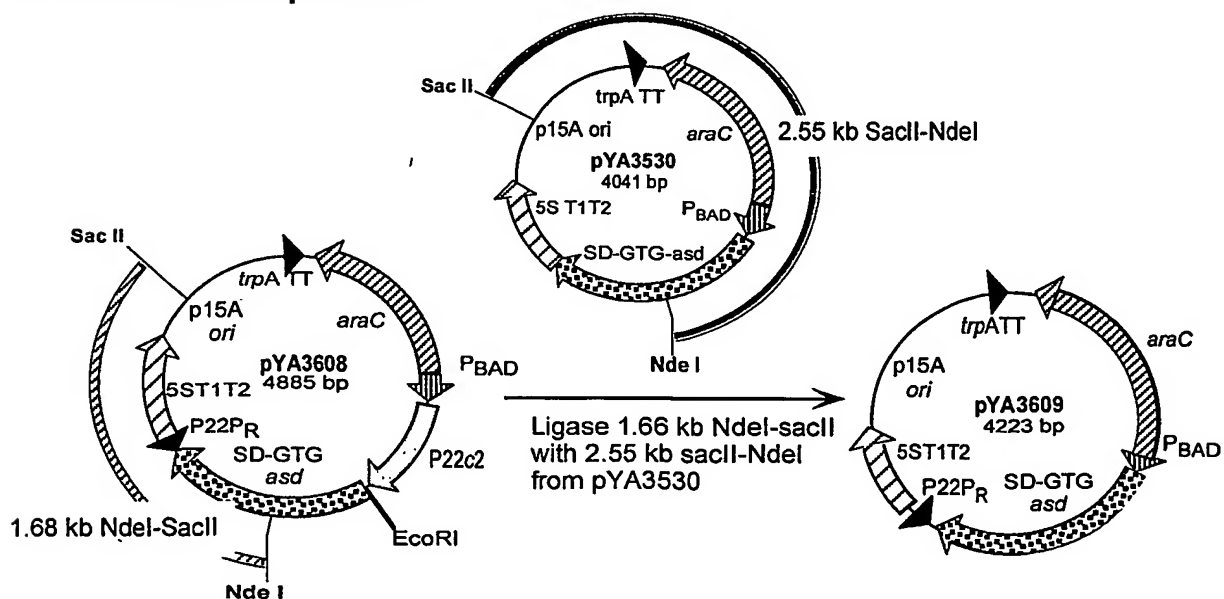
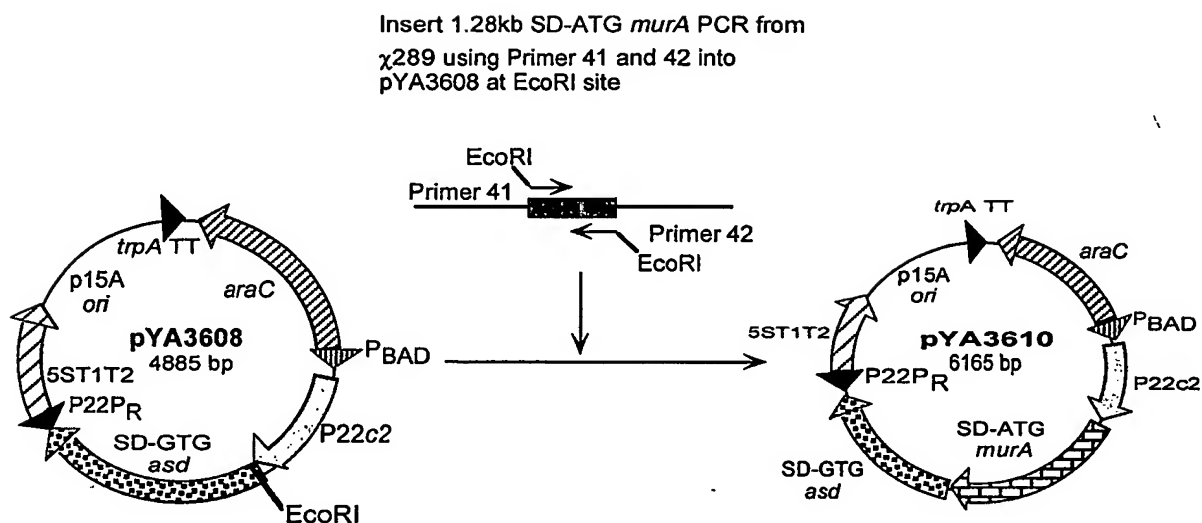


FIGURE 31. (continued)

C. Construction of pYA3610.



D. Construction of pYA3624.

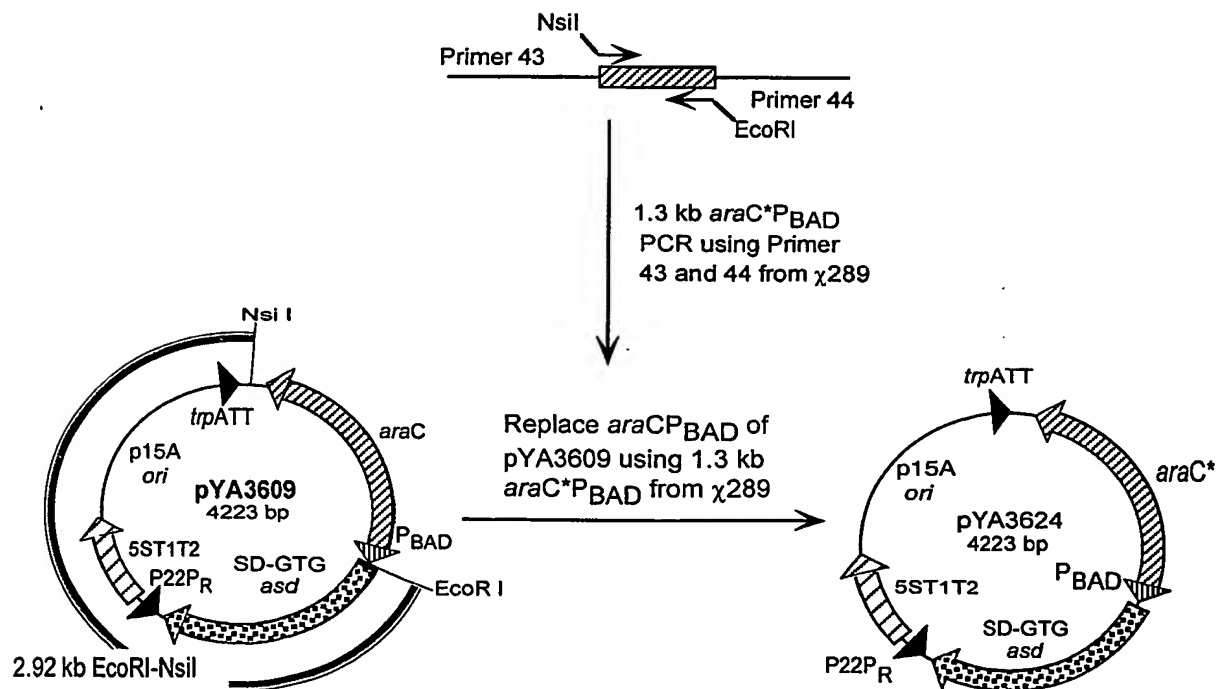
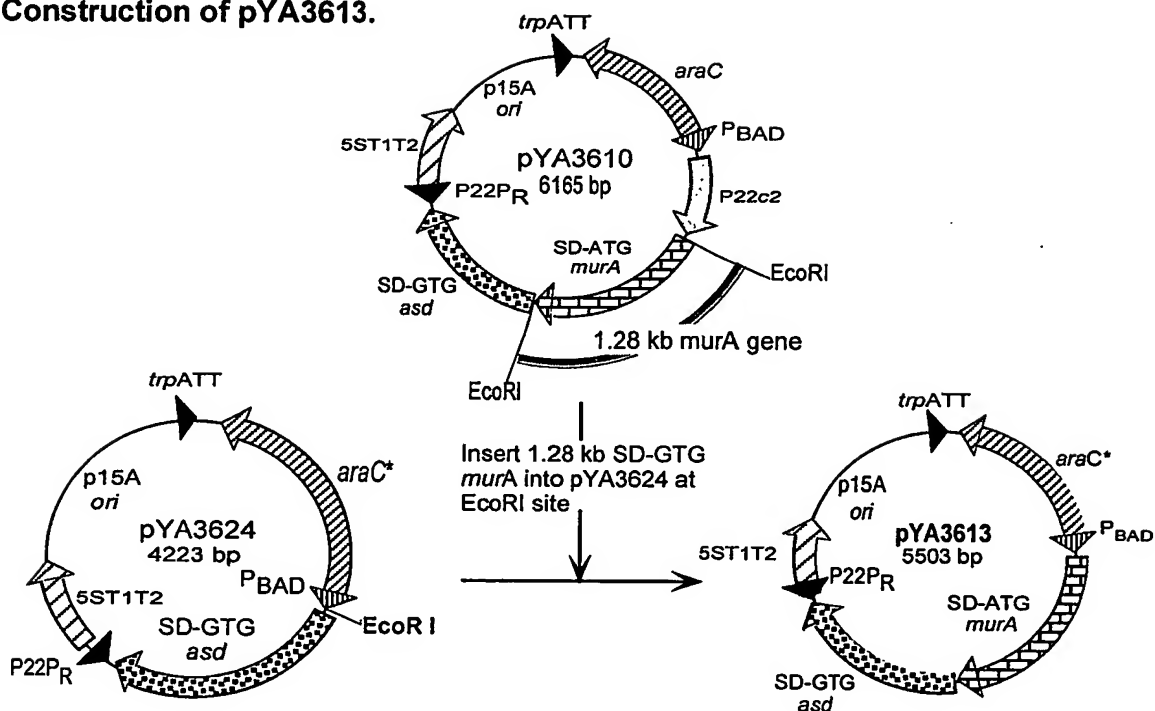


FIGURE 31. (continued)

E. Construction of pYA3613.



F. Construction of pYA3645.

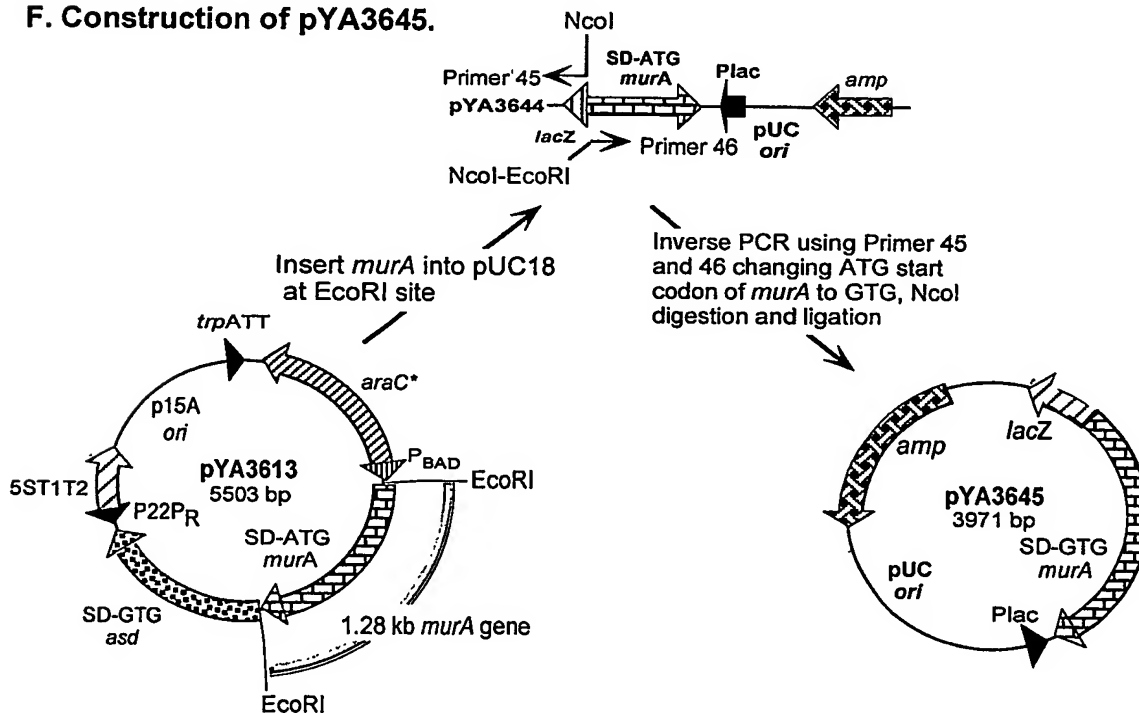


FIGURE 31. (continued)

G. Construction of pYA3646.

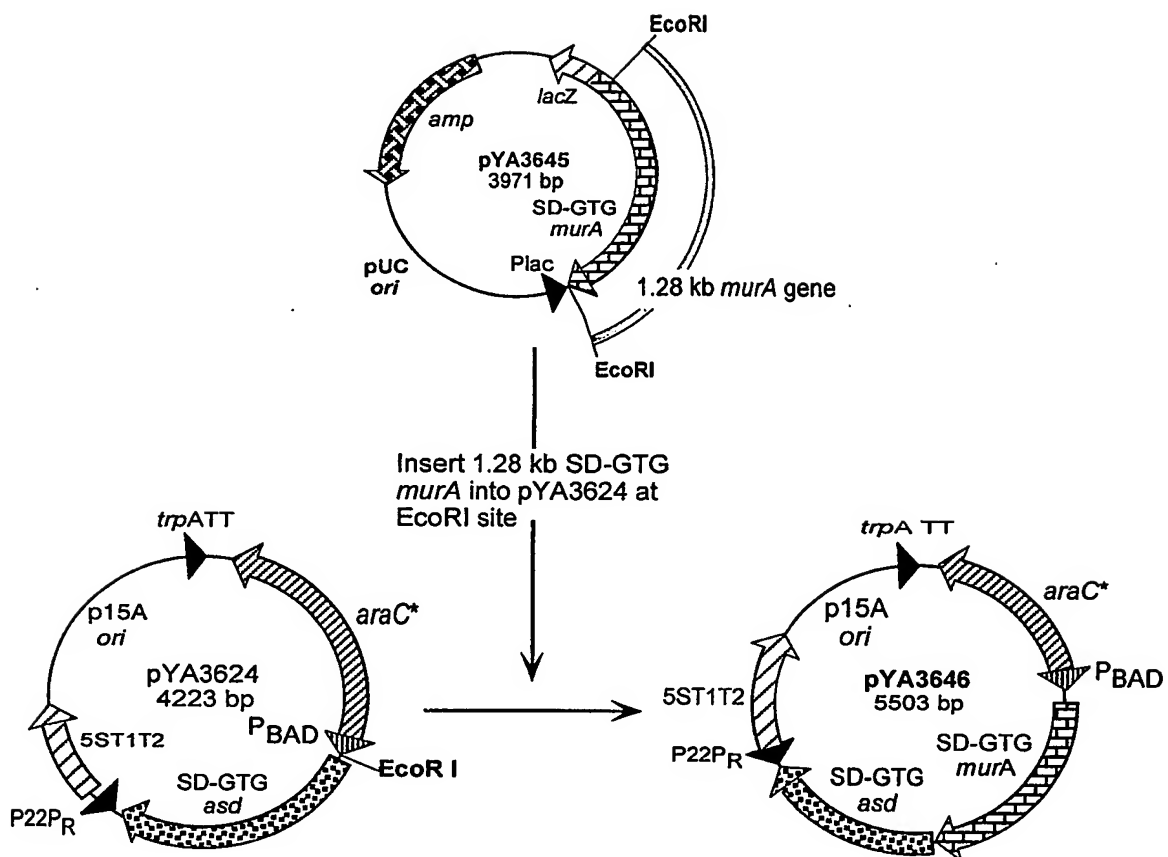


FIGURE 32. Cloning of *araC*P_{BAD} from *E.coli* K-12 to achieve tighter regulation and a lower level of transcription in the absence of arabinose than is achievable with the existing *araC*P_{BAD} system from *E.coli* B/r

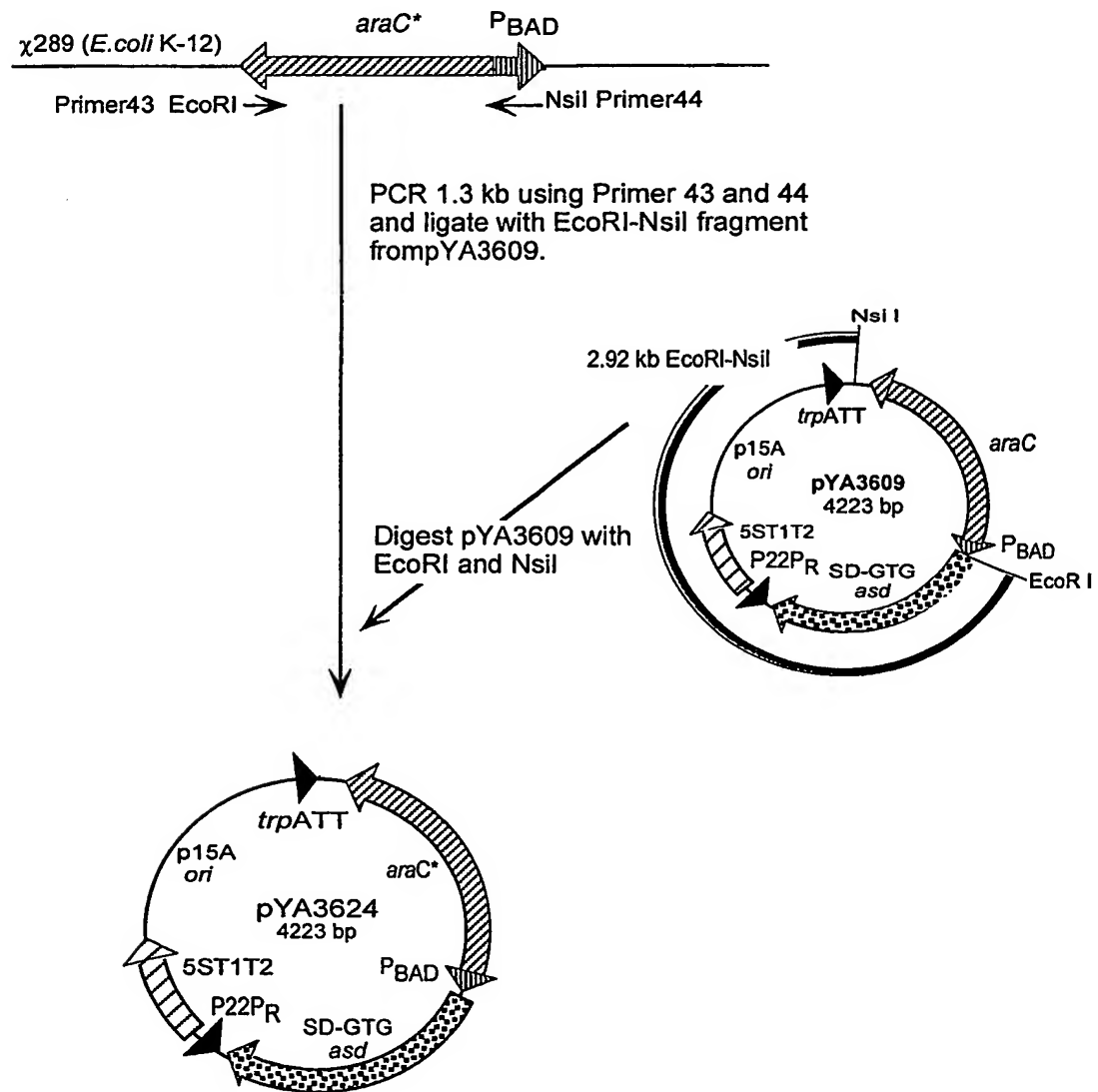


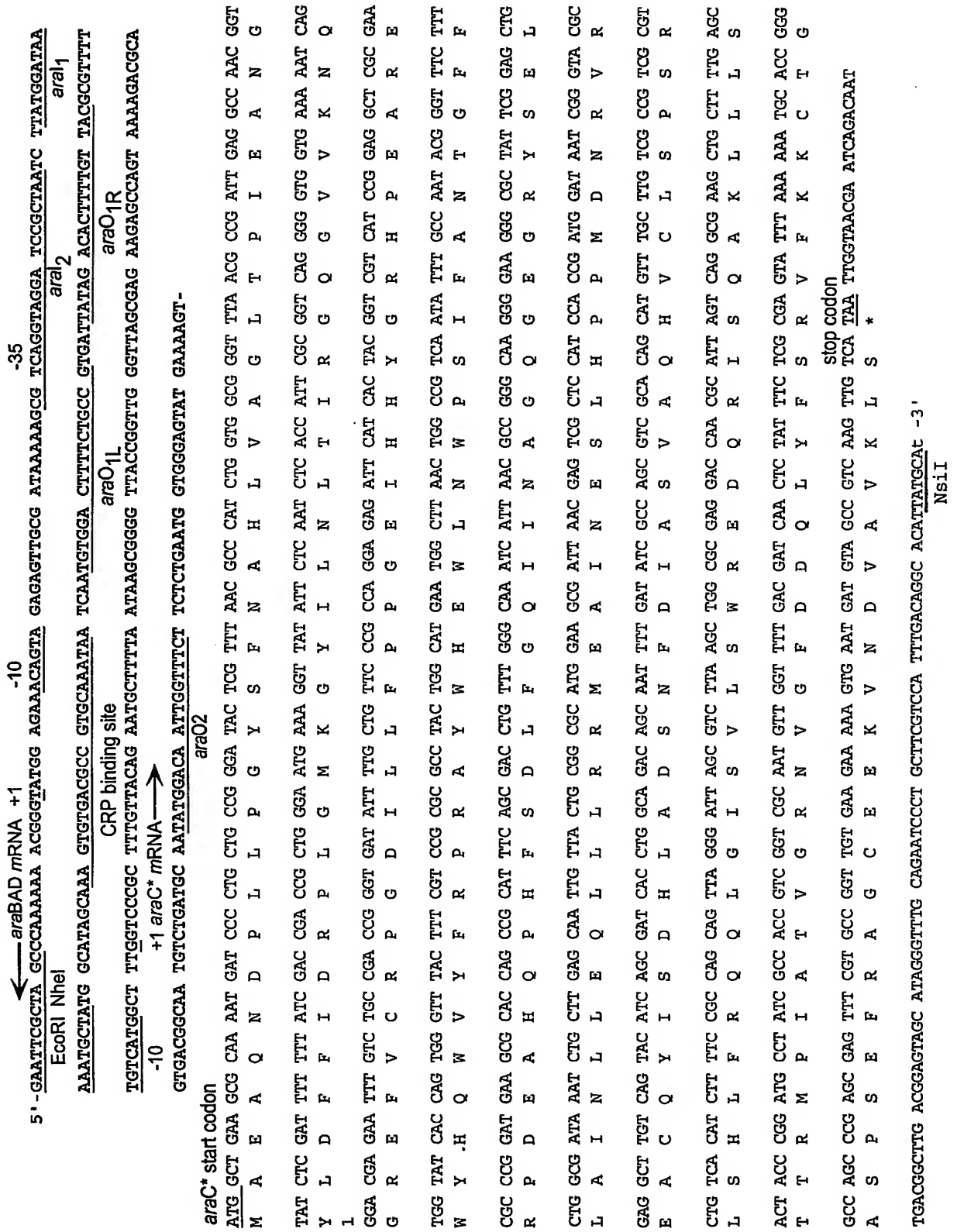
FIGURE 33. DNA nucleotide sequence of *araC**P_{BAD} region from χ 289 in pYA3624 and amino acid sequence of *AraC** protein

FIGURE 34. DNA nucleotide sequences alignment of the *E. coli* K-12 *araCP*^{BAD} region and the *E. coli* B/r *araCP*^{BAD} region

<i>E. coli</i> K-12	1	CCAAAAAACGGGTATGGAGAAACAGTAGAGATTGGGATAAAAAAGCGTCAGGTAGGATCCGCTAAATCTTATGGATAAAAAATGCTATGGCATAGCAAGT	100
<i>E. coli</i> B/r	1	CCAAAAAACGGGTATGGAGAAACAGTAGAGATTGGGATAAAAAAGCGTCAGGTAGGATCCGCTAAATCTTATGGATAAAAAATGCTATGGCATAGCAAGT	100
	101	GTGACGCGGTGCAAAATAATCAATGTGGACTTTTCTGCCGTGATTATAGACACTTTTGTACGCGTTTTTGTTCATGGCTTTGGTCCGCTTTGTACAGAA	200
	101	GTGACGCGGTGCAAAATAATCAATGTGGACTTTTCTGCCGTGATTATAGACACTTTTGTACGCGTTTTTGTTCATGGCTTTGGTCCGCTTTGTACAGAA	200
	201	TGCTTTTAAATAAGCGGGTTACCGGTTGGTTAGCGAGAAGAGCCAGTAAAGACGTCAGCGCAATGTCTGATGCAATATGAGACAATTTGGTTCTTC	300
	201	TGCTTTTAAATAAGCGGGTTACCGGTTGGTTAGCGAGAAGAGCCAGTAAAGACGTCAGCGCAATGTCTGATGCAATATGAGACAATTTGGTTCTTC	300
	301	TCTGAATGGTGGAGTAGAAAAAGTATGGCTGAAGCGCAAAATGATCCCTGTCTCCGGGATACCTCGTTAAAGCCCACTTGTGGCGGGTTTAAACGCCG	400
	301	TCTGAATGGCGGAGTAGAAAAAGTATGGCTGAAGCGCAAAATGATCCCTGTCTCCGGGATACCTCGTTAAAGCCCACTTGTGGCGGGTTTAAACGCCG	400
	401	ATTGAGGCCAACGGTTATCTCGATTTTATCGACCGACCGCTGGGAATGAAAGTTATTTCTCAATCTCACCATTTCGCGTCAAGGGGTGGTGAATA	500
	401	ATTGAGGCCAACGGTTATCTCGATTTTATCGACCGACCGCTGGGAATGAAAGTTATTTCTCAATCTCACCATTTCGCGTCAAGGGGTGGTGAATA	500
	501	ATCAGGACGAGAAATTTGTCTGCCGACCGGGTGAATTTTGTCTGTTCCCGCCAGGAGAGATTCTACTACGTCGTCTCATCCGGAGGCTCCGGAATGGTA	600
	501	ATCAGGACGAGAAATTTGTCTGCCGACCGGGTGAATTTTGTCTGTTCCCGCCAGGAGAGATTCTACTACGTCGTCTCATCCGGAGGCTCCGGAATGGTA	600

FIGURE 34. (continued)

E.coli K-12 601 TCACCAAGTGGGTTTACTTTTCGTCGCGCGCCTACTGGCAGTAAGTGGCTTAACCTGSCCGCTCAATATTGCGCAATACGGGTTCTTTTCGCCCGGATGAAGCG 700
|||||
601 TCACCAAGTGGGTTTACTTTTCGTCGCGCGCCTACTGGCAGTAAGTGGCTTAACCTGSCCGCTCAATATTGCGCAATACGGGTTCTTTTCGCCCGGATGAAGCG 700
620 640 660 680 700
720 740 760 780 800
701 CACCAGCGGCATTTTCAGCGACCTGTTTGGGCATATTAACGCCGGCAAGGGAGGGCGCTATTCCGAGCTGCTGGCGATAAATCTGCTTGAGCAAT 800
|||||
701 CACCAGCGGCATTTTCAGCGACCTGTTTGGGCATATTAACGCCGGCAAGGGAGGGCGCTATTCCGAGCTGCTGGCGATAAATCTGCTTGAGCAAT 800
720 740 760 780 800
801 TGTACTTGGCGGCATGGAAGCGATTAAACGAGTCGCTCCATCCACGATGGATAATCCGGTACGCGAGGCTTGTCAAGTACATCAGCCGATCACCTGGCAGA 900
|||||
801 TGTACTTGGCGGCATGGAAGCGATTAAACGAGTCGCTCCATCCACGATGGATAATCCGGTACGCGAGGCTTGTCAAGTACATCAGCCGATCACCTGGCAGA 900
820 840 860 880 900
920 940 960 980 1000
901 CAGCAATTTGATATCGCCAGCGTTCGACAGCATGTTTGTCTGTCGCGCTGCTGTCAATCTTTCCGCCAGGTTAGGGATTAGCGTCTTAAGC 1000
|||||
901 CAGCAATTTGATATCGCCAGCGTTCGACAGCATGTTTGTCTGTCGCGCTGCTGTCAATCTTTCCGCCAGGTTAGGGATTAGCGTCTTAAGC 1000
920 940 960 980 1000
1001 TGCGCGGAGGACCAACGATTAAGTCAGGGCAAGCTGTTTGGACATACCCCGGATGCCCTATCGCCACCGTCGGTCGCAATGTTGGTTTTCACGATCAAC 1100
|||||
1001 TGCGCGGAGGACCAACGATTAAGTCAGGGCAAGCTGTTTGGACATACCCCGGATGCCCTATCGCCACCGTCGGTCGCAATGTTGGTTTTCACGATCAAC 1100
1020 1040 1060 1080 1100
1101 TCTATTCTCGCGGTATTTAAATAATGACCCGGGCGAGCTGTTGCGCCGTTGTAAGAAAGTAATGATAGCCGTCAGCTTGC 1200
|||||
1101 TCTATTCTCGCGGTATTTAAATAATGACCCGGGCGAGCTGTTGCGCCGTTGTAAGAAAGTAATGATAGCCGTCAGCTTGC 1200
1120 1140 1160 1180 1200
1201 ATAAATGGTAAAGTAATGACCGCTTTCAGCGAGTAGCATAGGTTTGCAGAAATCCCTGCTTCCTCCATTGACAGGCACATTATGCA 1300
|||||
1201 ATAAATGGTAAAGTAATGACCGCTTTCAGCGAGTAGCATAGGTTTGCAGAAATCCCTGCTTCCTCCATTGACAGGCACATTATGCA 1300
1220 1240 1260 1280 1300

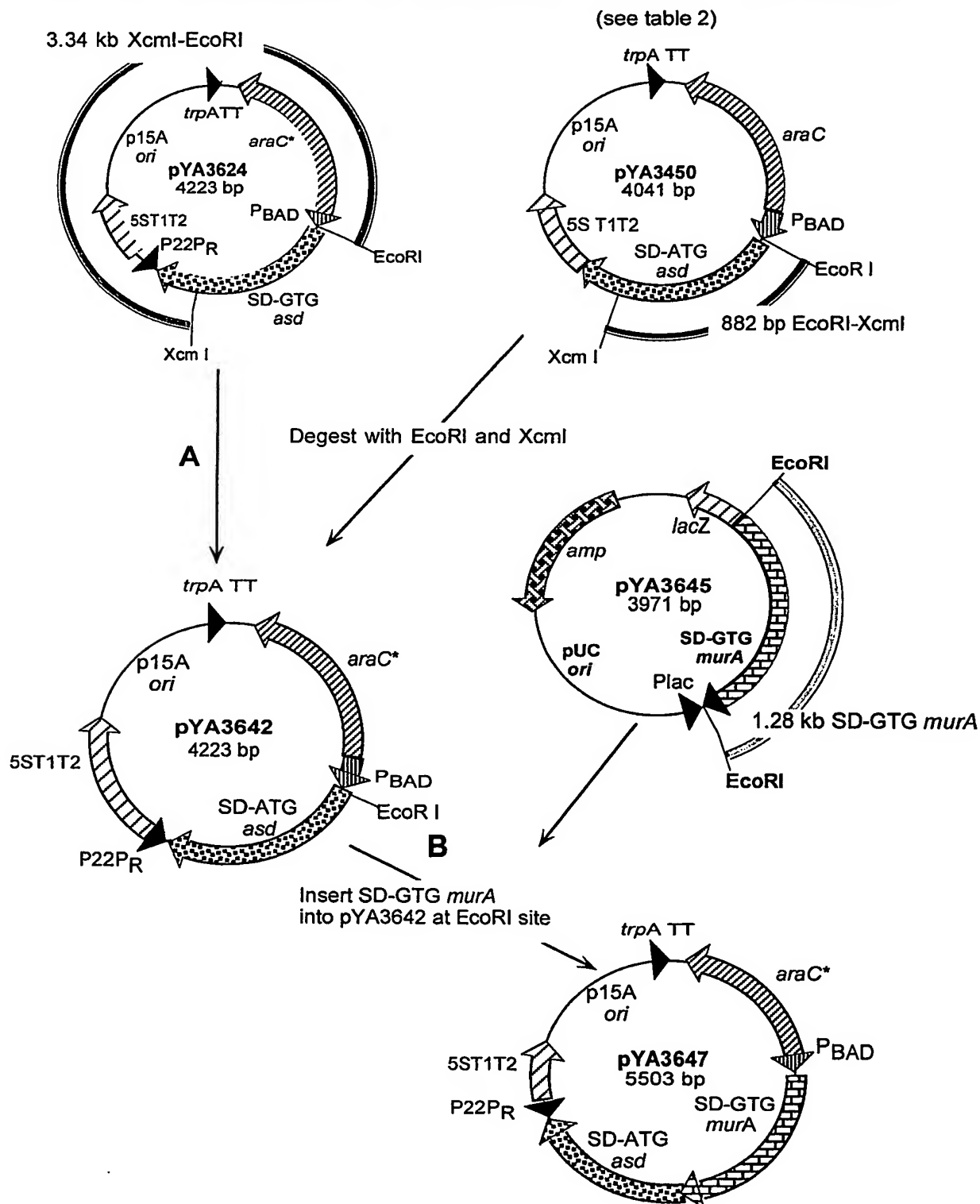
FIGURE 35. Construction of the regulatable lysis system vector pYA3647

FIGURE 36. Construction of the DNA vaccine vector pYA3650:

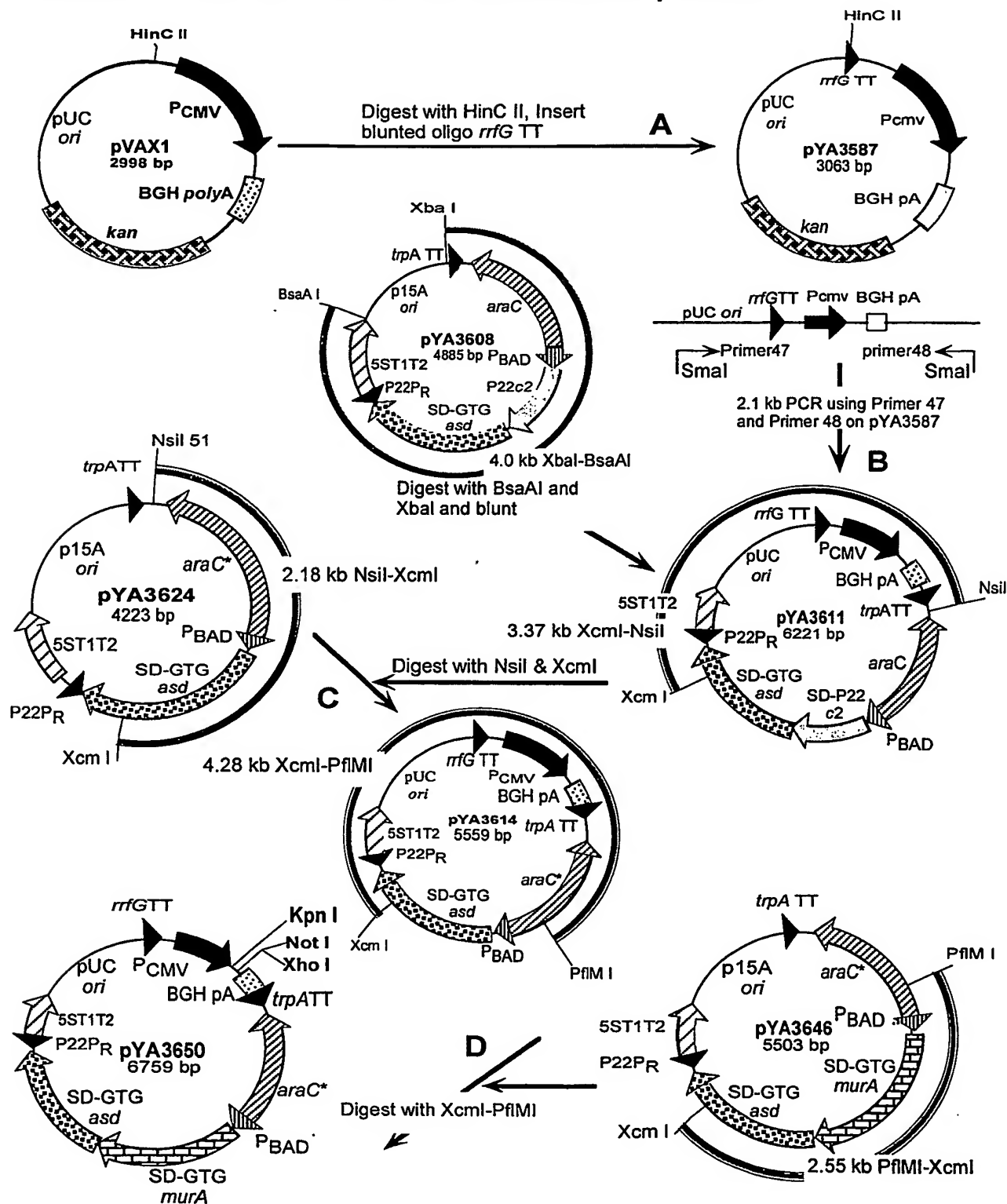
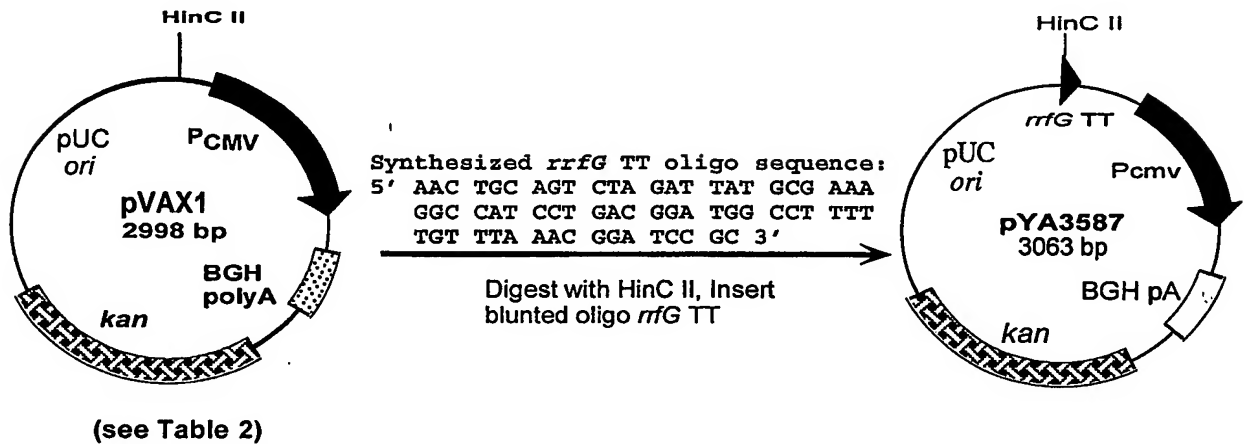


FIGURE 37. Steps in the construction of pYA3650

A. Construction of pYA3587.



B. Construction of pYA3611.

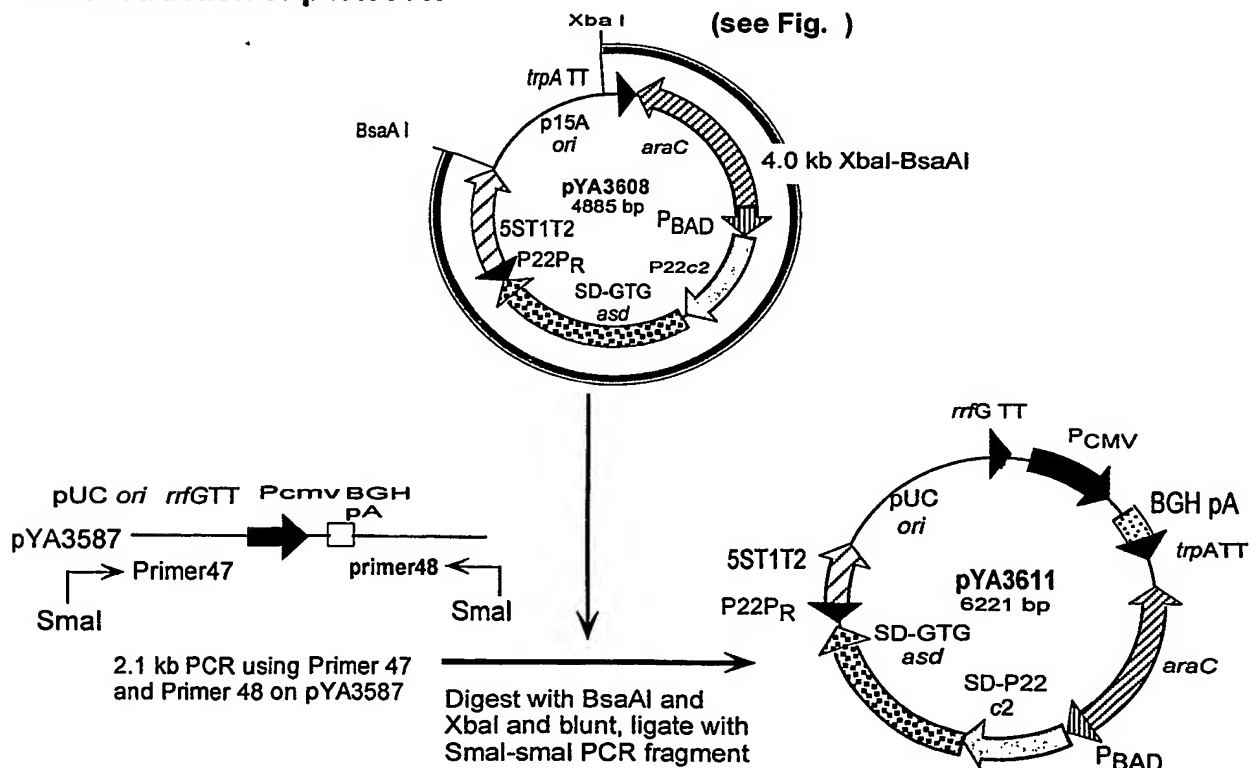
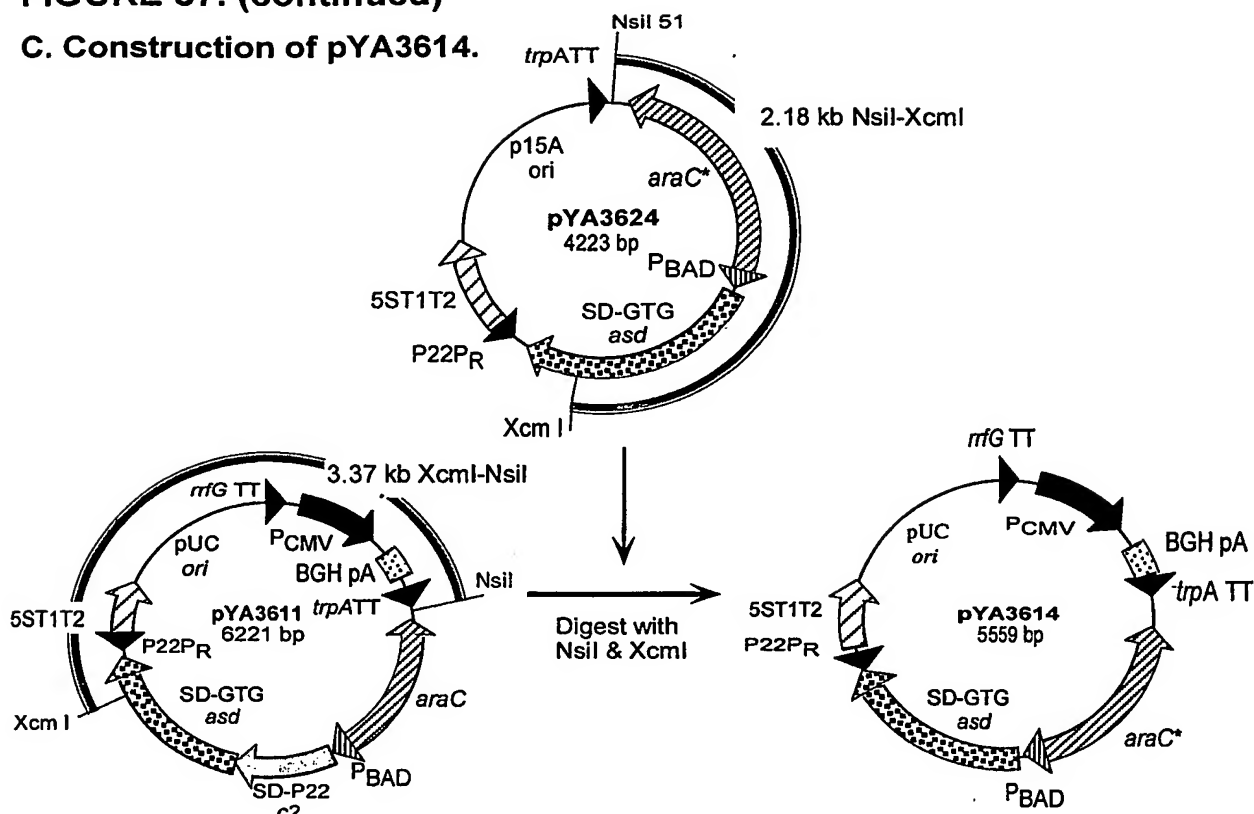


FIGURE 37. (continued)

C. Construction of pYA3614.



D. Construction of pYA3650.

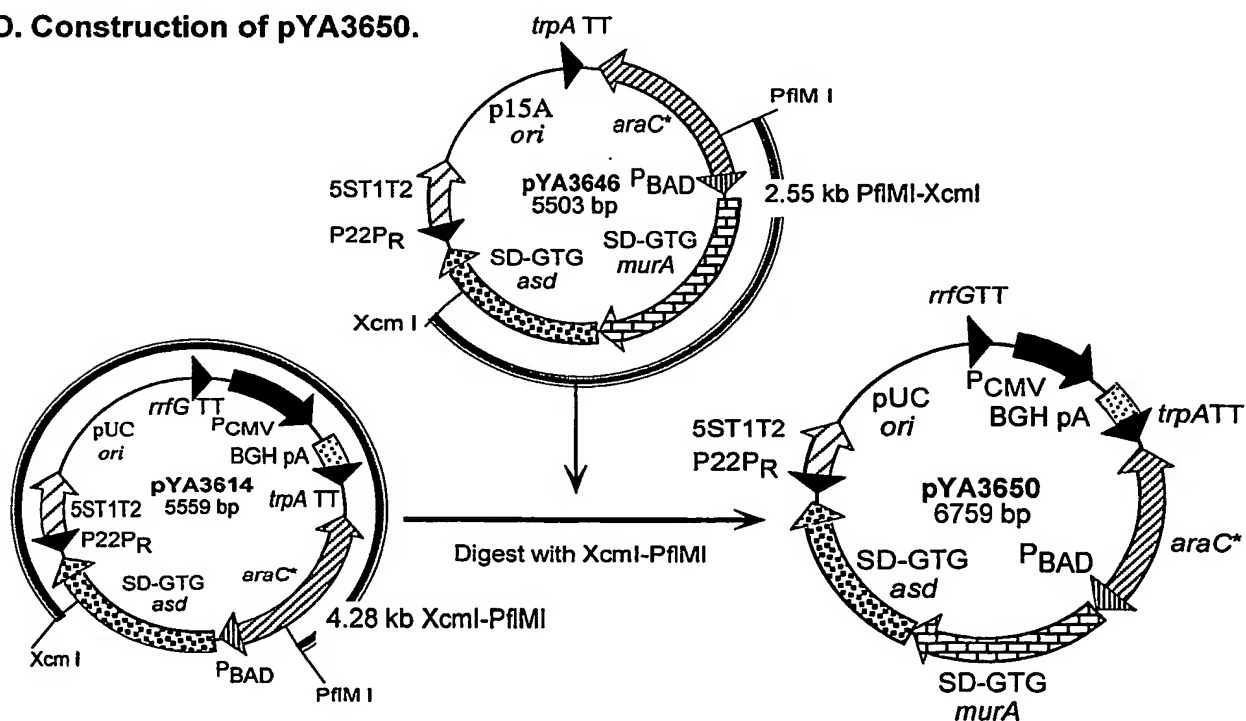


FIGURE 38. DNA sequence of DNA vaccine vector pYA3650 A. (1-3300 bp)

```

1  GACTCTTCGC GATGTACGG CCAGATATAC GGTAACTG CAGTCTAGAT TATGCCAAG GCCATCTGA CGGATGCGCT TTTGTTTAA ACGGATCCGC 100
101 GACATTGATT ATTGACTAGT TATTATAGT GGGGTATTA GTTCAGGCC CATATAGGA GTTCGCGT ACATACTTA CGGTAATGG 200
201 CCGCGCTGGC TGACCGCCCA ACGACCCCG CCCATTGAG TCAATTAAGA CGTATGTTCC CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG 300
301 GTGGACTATT TACGGTAAC TGCCCACTTG GCAGTACATC AAGTGATCA TATGCCAAGT ACGCCCTTA TTGACGTCAA TGACGGTAAA TGGCCCGCCT 400
401 GGCATTATGC CCAGTACATG ACCTTATGG ACTTCTCTAC TTGGCAGTAC ATCTAGTAT TAGTCATGC TATTACCATG GTGATCGGT TTTGGCAGTA 500
501 CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCATATGA CGTCAATGGG AGTTTGT TTTT GGCCACAAA TCAACGGGAC 600
601 TTTCAAAAAT GTCGTAAACA CTCGGCCCA TTGACGCAA TTGACGTAAGT GGGGAGTCT ATATAAGCAG AGTCTCTGG CTAACGTAGAG 700
701 AACCACATGC TTACTGGCTT ATCGAAATTA ATACGACTCA CTATAGGGAG ACCCAAGCTG GCTAGCGTTT AAACTTAAGC TTGGTACOGA GCTCGGATCC 800
801 ACTAGTCCAG TGTGTGGAA TTCTGCAGAT ATCCAGACA GTGGCGGCGG CTCGAGTCTA GAGGGCCCGT TTAACCCCGC TGATCAGCCT CGACTGTGCC 900
901 TTCTAGTTGC CAGCCATCTG TGTGTTGCC GTTCTGATCT GTGCTAGTAG GTTCTGGG GTGGGTGGG GCAGGACAGC AAGGGGAGG ATTGGAAGA CAATAGCAGG CATGCTGGGG 1100
1001 GCATCGCATT GTCTGAGTAG GTGCTATCT GTTCTGAGT TGTGTTGAAG TCTAGCCCG CTAATGAGCG GCGTGTGCA ATTTATCTCT TCNAATGTAG 1200
1101 ATGCGGTGGG CTCTATGGCT TCTATGGCT TGTGTTGAAG TCTAGCCCG CTAATGAGCG GCGTGTGCA ATTTATCTCT TCNAATGTAG 1300
1201 CACCTGAAGT CAGCCCATCA CGATATAAGT TGTGTTGAAG TCTAGCCCG CTAATGAGCG GCGTGTGCA ATTTATCTCT TCNAATGTAG 1400
1301 CTGTCAATG GACGAAGCAG GGAATCTGCA AACCTATGCT TACTCGCTCA AGCCGTCAT GTCTGATATC GTTACCAAT ATGACAACTT GACGGCTACA 1500
1401 TCATTCATTT TTCTTCAACA ACCGGCAGCA AACTCGTCTG GGTGCGCCC GGTGCAATTT TTAATFACCT GCGAGAAATY GAGTTGATCG TCNAACCAA 1600
1501 CATTCGAC GACGGTGGC ATAGGCATCC GGTGATGCT CAAAGCAGC TTGCGCTGAC TAATGCGTTG GTCTCGCGC CAGCTTAAGA CGTAAATCCC 1700
1601 TAACTGCTGG CGRAAAGAT GTGACAGAGC CGACGGGAC AAGCAACAT GCTGTGCGAC GCTGCGEATA TCAAAATGTC TGTCTGCCAG GTGATCGCTG 1800
1701 ATGTACTGAC AAGCTTCGCG TACCGGATTA TCCATCGGTG GATGGAGCGA CTCGTTAATC GCTTCATG CCGCAGTAA CAATTGCTCA AGCAGATTTA 1900
1801 TCGCCAGCAG CTCGGAATAG CGCCCTTCCC CTTGCGCGG GTTAATGATT TGCCCAACA GGTGCTGAA ATGCGGCTGG TGCGCTTCAT CCGGGCGAAA 2000
1901 GAAACCGTA TTGGCAATA TTGACGGCCA GTTAAGCCAT TCATGCCAGT AGGCGCGCG ACRAAGTAA ACCCACTGGT GATACCAATC GCGAGCTCC 2100
2001 GGATGACGAC CGTAGTGATG AATCTCTCT GCGCGGAACA GCAAAATATC ACCCGTCCG CAGACAAAT CTOGTCCCTG ATTTTTCACC ACCCCTGAC 2200
2101 CGCGAATGGT GAGATTGAGA ATATAACCTT TCATTCCCAG CGGTGCGTGC ATAAAAAT CGAGATAACC GTTGGCTCA ATCGGCGTTA AACCGGCCAC 2300
2201 CAGATGGCGG TTAACGAGT ATCCCGGCG CAGGGGATCA TTTTGGGCTT CAGCCATATC TTTCATPACT CCACCATCA GAGAGAAAC CAATTGTCCA 2400
2301 TATTGATCA GACATTGCCG TCATGCGTC TTTTACTGGC TTTTACTGGA AAAGTCCACA TTGATATTT GCACGCGCTC ACATTTGCT ATGCCATAGC 2500
2401 CCAAGCCAT GACAAAACG CGTAACAAA GTGTCTATAA TCACGCGAGA AAGTCCACA TTTGATATTT GCACGCGCTC ACATTTGCT ATGCCATAGC 2600
2501 ATTTTATACC ATAAGATTAG CGGATCCTAC CTGAGCTTT TTATGCAAC TCTCTACTGT TTCTCATATC CCGTTTTTTT GGGCTAGCGA ATCTGAGAA 2700
2601 CAACTAAGT GGATAAATTT CGTGTTCAGG GGCCACGAA GCTCCAGGGC GAATCACA TTTCCGGCGC TAAATGCT GCTCTGCTA TCCTTTTTC 2800
2701 CGCACTACTG GCGGAAGAC CCGTAGAGAT CCAGAAGCTC CCGAAGCTGA AAGAGTCCA TACATCAATG AAGTGTCTAA GCCAGTGGG TGCAGAACTA 2900
2801 GAACGTAATG GTTCTGTGCA TATTGATGCC CCGGAGCTTA ATGTATCTG CGCACTTAC GATCTGGTTA AAACCTATC TGCTTCTATC TGGCGCTGG 3000
2901 GGCCTGTGTT AGCGCGCTTT GGTGAGGGG AAGTTTCACT ACCTGGCGGT TGTAGATCG GTGCGGTCC CATTGATCTA GCAATTTCTG GCCTCGAACA 3100
3001 ATTAGCGCG ACCATCAAC TGAAGAAG TTACGTTAAA GCTTCGCTG ATGTCGTTT GAAAGTGTGA CATATCGTGA TGGATAAAGT CAGCGTTGGC 3200
3101 GCAACGGTGA CATCATGTG TGCTGCAAC CCACGAGAG GCACCAAGT TATTGAAAC GCAGCGCTG AACCGGAAAT CGTCGATACC GCGAACTTCC 3300
3201 TGATTAGCT GGTGCGAAA ATTAGCGGT ATCGAAGGTG TGGAAAGTTT TGGAAAGTTT AGGCGGCGGT GTCTATCGCG TTCTGCGCGA

```

FIGURE 38. (continued) DNA sequence of DNA vaccine vector pYA3650 B. (3301- 6759bp)

3301 TCGTATCGAA ACCGGTACTT TCCTGGTGGC GCGGCGGATT TCCTGGCGGA AATTATCTG CCGTAACGGC CAGCCAGATA CTCTCGACGC CGTCTGGCG 3400
 3401 AAACGCGTG AGCTGGAGC GGACATCGAA GTCCGGCGAAG ACTGGATTAG CTTGGATATG CATGCCAAAC GTCCGAAAGC TGTAAACGTA CGTACCGCGC 3500
 3501 CGCATCCGC ATTCGCCAGC GATATGCAGG CCAGTTCAC GCTGTTGAAC CTGGTGGCAG AAGGACCGG GTTATATCAC GAAACGGTCT TTGAAAACCG 3600
 3601 CTTTATGCAT GTGCCAGAC TGAGCCGTAT GCGCGCGCAC GCGGAAATCG AAAGCAATAC CGTTATTTGT CACGGTGTG AAAAACCTTC TGGCGCACAG 3700
 3701 GTTATGGCAA CCGATCTGC TGATCAGCA AGCTGTGTC TGTGCTGGTG TATGCGGAA GGTAGACAGG TGGTGTATCG TATTTATCAC ATCGATCGTG 3800
 3801 GCTACGAACG CATTGAAGC AATGTCGCG AATGTCGCG CTTTAGTTC AATATTGAG CGTGTGAAAG GCGAATAAGA ATTACGAAA AAAACGCTGT GAAAAATGTT 3900
 3901 GGTATTATCG GCTGGCGCG AATGTCGCG CCACTTCG AATGTCGCG CTTTATCTCA TGCAACGAT GTTAGAGGAG CCGGATTTG ACCTGTTTC TTTTCTACCT 4000
 4001 CCCAGTTGG ACAGCGCGG CCACTTCG AATGTCGCG CCACTTCG CCACTTCG CTTTATCTCA TGCAACGAT GTTAGAGGAG CCGGATTTG ACCTGTTTC TTTTCTACCT 4100
 4101 GACCTGCCAG GCGCGCGG AATGTCGCG CCACTTCG AATGTCGCG CCACTTCG CCACTTCG CTTTATCTCA TGCAACGAT GTTAGAGGAG CCGGATTTG ACCTGTTTC TTTTCTACCT 4200
 4201 AAAGATGAT CCAATTATT TCTCGACCG GTCAACCCAG ACCTGATAC CAGCGGCTG AACATGGCG TGAAGACCTT TGTGGGGGT AACTGTACCG 4300
 4301 TTAGCTGAT GTTGATGTC CTGGGCGGT CTTTGGGTC TCACTGCTT GACTGGGTAT CCGTCCGCG CTATCAGGCC CCGTCCGCG CCGCGCGCG 4400
 4401 CCAATGCGC GAGCTGTTA CCAGATGGG TCACTGCTT GACTGGGTAT CCGTCCGCG CTATCAGGCC CCGTCCGCG CCGCGCGCG TGAACGCAA 4500
 4501 GTTACGGCAT TGACCGCGC CCGCGCGC CCGTGGGT ACTTGGGT ACTTGGGT ACCGCTGGG GGAAGCCTGA TCCCTGGAT CCGTCCGCG 4600
 4601 GCGAGAGCG CGAAGAGTG AAGGCGCAG CCGAATCTC CAAGTCTC AATGCTGC CTTGATTTG GGTGATTTG TGTGCTGC CCGTCCGCG 4700
 4701 GCTGCGTGT CACAGCCAG CGTTCACCAT CAAGCTGAAA AAGAGGAT CCACTCCGAC GGTGGAAGAA CTGCTGGCG CACATAATCC GTGGCGAAA 4800
 4801 GTGCTGCCA ACGATCGTA TATCACTATG CCGGAATTA CCGCGCGCG GTGACCCGC ACCTGACTA CCGCGGTGG TCGTCTGCGT AAGCTGAACA 4900
 4901 TGGGCGCAGA GTTCTGTG CCGTGTACCG TAGGCGACCA GTTGTATGG GCGCGCGCG AGCGCTGG TCGAATGCTG CGCCAGTTGG CGTAGCTAG 5000
 5001 CTGACGATA CCGTGCAT GTACATAGC TCGTCCGAA ATTAAAGAA ACTTAAATA TCTACTAAAG GAATCTTTAG TCAAGTTAT TTAAGATGAC 5100
 5101 TTAACATGA ATACAAAT GTAGGATGT CCGTATGAT TCCATTATG AAGCATTTAT CAGGTTTAT CAGGTTTAT GTCTCATGAG CTTGGCTGTT TTGGCGGATG 5200
 5201 AGAAGAGAT TTAGCCTGA TACAGATTAA ATCAGAACG AGAAGCGGT TCGAATAACA GTTGGCTGG GGTGCTGGG CACCTGACCC 5300
 5301 CATGCCGAC TCAGAGTGA AAGCGGTAG CCGCGGTAT AGTGTGGGT CTCCCATGC GAGAGTAGG AACTGCCAG CATCAATAA AACGAAAGC 5400
 5401 TCAGTGGAA GACTGGGCT TTGCTTTTAT CCGTGTGAT TCGGTGAAG CTCTCTGAG TAGGACAAAT CCGCGGGAG CCGAATTGAA CGTTGCGAAG 5500
 5501 CAACGGCCG GAGGTGGC GGCAGGACG CCGCCATAA CTGCCAGCA TCAATTAAG CAGAAGGCA TCCGTGACGA TGGCTTTT GCGTTCTTAT 5600
 5601 AAACCTCTT TGTATTAT TCTAATACA TCTAATATG TATCCGCTCA TGAGACAATA ACCCTGATA ATGCTTCAAT AATGGAAGAT CTTCCAACAT 5700
 5701 CACAGGTAA CAGAAACGTC GGTGCGATC GGAATCTT TCCCGACCG GCGGGGTG GCAAGCCGC AGCGCGTCA GTGCTTTAG CCGGTGTCG 5800
 5801 GCGAGCCCTG AACAGTCA GGTATCGAT TGTGCGGTAT TTCAACCCG ATACAGGTG GCAATTTGCG GGAATGTC GCGGAACCC TATTTGTTA 5900
 5901 TTTTCTTAA TACATTCAA TATGATCCG CTCATGAGC AATAACCTG AATAACCTG AATAACCTG AATAACCTG AATAACCTG AATAACCTG 6000
 6001 GATCTAGT GAATCTCT TTTGATAATC TCATGACCA AATCCCTTAA CGTGATTTT CTTTCCACTG AGCTCAGAC CCGTAGAAA AGATCAAAG 6100
 6101 ATCTCTTGA GATCTCTT TTTCTGCGGT AATCTGCTG TTGCAACAA AAAAACCC GCTACACCG GTGGTTTGT TGGCGGATCA AGACTACCA 6200
 6201 ACTCTTTTC CAAAGGTAA TGGCTTCAG TGGCTTCAG TGGCTTCAG TGGCTTCAG TGGCTTCAG TGGCTTCAG TGGCTTCAG TGGCTTCAG 6300
 6301 CACCGCTAC ATACCTCTGTTT CTTGATCTGTTT CTTGATCTGTTT CTTGATCTGTTT CTTGATCTGTTT CTTGATCTGTTT CTTGATCTGTTT CTTGATCTGTTT 6400
 6401 GATGAGGCG CAGCGCTCG GTGATCTGTTT CTTGATCTGTTT CTTGATCTGTTT CTTGATCTGTTT CTTGATCTGTTT CTTGATCTGTTT CTTGATCTGTTT 6500
 6501 TGAGAAAGC CCAAGCTTCC CCAAGCTTCC CCAAGCTTCC CCAAGCTTCC CCAAGCTTCC CCAAGCTTCC CCAAGCTTCC CCAAGCTTCC 6600
 6601 ACGCTGCTA TCTTTATAGT CTTGCTGGGT TTTGCTGGGT TTTGCTGGGT TTTGCTGGGT TTTGCTGGGT TTTGCTGGGT TTTGCTGGGT 6700
 6701 CAGCAACCG GCCTTTTATC GCTTTGCTG CTTTGTGCTC ACATGTTCT 6759

FIGURE 39. Oligo nucleotide sequence of synthesized *rrfG* TT and multiple cloning site of pYA3650

Synthesized *rrfG* TT oligo sequence:

5' AAC TGC AGT CTA GAT TAT GCG AAA GGC CAT CCT GAC
GGA TGG CCT TTT TGT TTA AAC GGA TCC GC 3'

Multiple Cloning Site of pYA3650:

AATTAATACG ACTCACTATA GGGAGACCCA AGCTGGCTAG CGTTTAAACT
T7 promoter/priming site

TAAGCTTGGT ACCGAGCTCG GATCCACTAG TCCAGTGTGG TGAATTCTG
KpnI

CAGATATCCA GCACAGTGGC GGCCGCTCGA GTCTAGAGGG CCCGTTTAAA
NotI XhoI Stop codon

CCCGCTGATC AGCCTCGACT GTGCCTTCTA GTTGCCAGCC ATCTGTTGTT

TGCCCCCTCCC CCGTGCCTTC CTTGACCCTG GAAGGTGCCA CTCCCCTGT

CCTTTCCTAA TAAAATGAGG AAATTGCATC
BGH poly A signal

FIGURE 40. DNA and amino acid sequences of *GTG-murA* gene of pYA3650

1/1
 GTG GAT AAA TTT CGT GTT CAG GGG CCA ACG AAG CTC CAG GGC GAA GTC ACA ATT TCC GGC GCT AAA AAT GCT GGT CTG CCT ATC CTT TTT
 M D K F R V Q G P T K L Q G E V T I S G A K N A A L P I L F
 91/31
 GCC GCA CTA CTG GCG GAA GAA CCG GTA GAG ATC CAG AAC GTC CCG AAA CTG AAA GAC GTC GAT ACA TCA ATG AAG CTG CTA AGC CAG CTG
 A A L L A E E P V E I Q N V P K L K D V D T S M K L L S Q L
 181/61
 GGT GCG AAA GTA GAA CGT AAT GGT TCT GTG CAT ATT GAT GCC GGC GAC GTT AAT GTA TTC TGC GCA CCT TAC GAT CTG GTT AAA ACC ATG
 G A K V E R N G S V H I D A R D V N V F C A P Y D L V K T M
 271/91
 CGT GCT TCT ATC TGG GCG CTG GGG CCG CTG GCG GCG TTT GGT CAG GGG CAA GTT TCA CTA CCT GGC GGT TGT ACG ATC GGT GCG CGT
 R A S I W A L G P L V A R F G Q G Q V S L P G C T I G A R
 361/121
 CCG GTT GAT CTA CAC ATT TCT GGC CTC GAA CAA TTA GGC GCG ACC ATC AAA CTG GAA GAA GGT TAC GTT AAA GCT TCC GTC GAT GGT CGT
 P V D L H I S G L E Q L G A T I K L E E G Y V K A S V D G R
 451/151
 TTG AAA GGT GCA CAT ATC GTG ATG AAT GTC AGC GTT GGC GCA ACG GTG ACC ATC ATG TGT GCT GCA ACC CTG GCG GAA GGC ACC ACG
 L K G A H I V M D K V S V G A T V T I M C A A T L A E G T T
 541/181
 ATT ATT GAA AAC GCA GCG CGT GAA CCG GAA ATC GTC GAT ACC GCG AAC TTC CTG ATT ACG CTG GGT GCG AAA ATT AGC GGT CAG GGC ACC
 I I E N A A R E P E I V D T A N F L I T L G A K I S G Q G T
 631/211
 GAT CGT ATC GTC ATC GAA GGT GTG GAA CGT TTA GGC GGC GGT GTC TAT CGC GTT CTG CCG GAT CGT ATC GAA ACC GGT ACT TTC CTG GTG
 D R I V I E G V E R L G G G G V Y R V L P D R I E T G T F L V
 721/241
 GCG GCG CCG ATT TCT CCG GGC AAA ATT ATC TGC CGT AAC CCG CAG CCA GAT ACT CTC GAC GCC GTG CTG CCG AAA CTG GGT GAC GCT GGA
 A A A I S R G K I I C R N A Q P D T L D A V L A K L R D A G
 811/271
 CCG GAC ATC GAA GTC GGC GAA GAC TGG ATT AGC CTG GAT ATG CAT GGC AAA CGT CCG AAG GCT GTT AAC GTA CGT ACC GCG CCG CAT CCG
 A D I E V G E D W I S L L D M H G K R P K A V N V R T A P H P
 901/301
 GCA TTC CCG ACC GAT ATG CAG GCC CAG TTC ACG CTG TTG AAC CTG GCA GAA GGG ACC GGG TTT ATC ACC GAA ACG GTC TTT GAA AAC
 A F P T D M Q A Q F T L L N L V A E G T G F I T E T V F E N
 991/331
 CGC TTT ATG CAT GTG CCA GAG CTG AGC CGT ATG GGC CCG CAC GCC GAA ATC GAA AGC AAT ACC GTT ATT TGT CAC GGT GTT GAA AAA CTT
 R F M H V P E L S R M G A H A E I E S N T V I C H G V E K L
 1081/361
 TCT GGC GCA CAG GTT ATG GCA ACC GAT CTG GGT GCA TCA GCA AGC CTG GTG CTG GCT GGC TGT ATT CCG GAA GGG ACG ACG GTG GTT GAT
 S G A Q V M A T D L R A S A S L V L A G C I A E G T T V V D
 1171/391
 CGT ATT TAT CAC ATC GAT CGT GGC TAC GAA CGC ATT GAA GAC AAA CTG CGC GCT TTA GGT GCA AAT ATT GAG CGT GTG AAA GGC GAA TAA
 R I Y H I D R G Y E R I E D K L R A L G A N I E R V K G E *

FIGURE 41. DNA and amino acid sequences of GTG-asd gene of pYA3650

1/1
 GTG AAA AAT GTT GGT TTT ATC GGC TGG CGC GGA ATG GTC GGC TCT GTT CTC ATG CAA CGC ATG GTA GAG GAG CGC GAT TTC GAC GCT ATT
 M K N V G G F I G W R CGC GGA ATG GTC GGC TCT GTT CTC ATG CAA CGC ATG GTA GAG GAG CGC GAT TTC GAC GCT ATT
 91/31
 CGC CCT GTT TTC TTT TCT ACC TCC CAG TTT GGA CAG GCG GCG CCC ACC TTC GGC GAC ACC TCC ACC GGC ACG CTA CAG GAC GCT TTT GAT
 R P V F F S T S Q F G Q A A P T T F G D T S T G T L Q D A F D
 181/61
 CTG GAT GCG CTA AAA GCG CTC GAT ATC ATC GTC ACC TGC CAG GGC GGC GAT TAT ACC AAC GAA ATT TAT CCA AAG CTG CGC GAA AGC GGA
 L D A L K A L D I I V T C Q G G D Y T N E I Y P K L R E S G
 271/91
 TGG CAG GGT TAC TGG ATT GAT GCG GCT TCT ACG CTG CGC ATG AAA GAT GAT GCC ATT ATT ATT CTC GAC CGC GTC AAC CAG GAC GTG ATT
 W Q G Y W I D A A S T L R M K D D A I I I L D P V N Q D V I
 361/121
 ACC GAC GGC CTG AAC AAT GGC GTG AAG ACC TTT GTG GGC GGT AAC TGT ACC GTT AGC CTG ATG TTG ATG TCG CTG GGC GGT CTC TTT GCC
 T D G L N N G V K T F V G G N C T V S L M L M S L G G L F A
 451/151
 CAT AAT CTC GTT GAC TGG GTA TCC GTC GCG ACC TAT CAG GCC GCC TCC GGC GGC GCG CAT ATG CGC GAG CTG TTA ACC CAG ATG
 H N L V D W V S V A T Y Q A A S G G A R H M R E L L T Q M
 541/181
 GGT CAG TTG TAT GGC CAT GTC GCC GAT GAA CTG GCG ACG CCG TCT TCC GCA ATT CTT GAT ATT GAA CGC AAA GTT ACG GCA TTG ACC CGC
 G Q L Y G H V A D E L A T P S S A I L D I E R K V T A L T R
 631/211
 AGC GGC GAG CTG CCG GTT GAT AAC TTT GGC GTA CCG CTG GCG GGA AGC CTG ATC CCC TGG ATC GAC AAA CAG CTC GAT AAC GGC CAG AGC
 S G E L P V D N F G V P L A G S L I P W I D K Q L D N G Q S
 721/241
 CGC GAA GAG TGG AAA GGC CAG GCG GAA ACC AAC AAG ATT CTC AAT ACT GCC TCT GTG ATT CCG GTT GAT GGT TTG TGT GTG CGC GTC GGC
 R E E W K G Q A E T N K I L N T A S V I P V D G L C V R V G
 811/271
 GCG CTG CCG TGT CAC AGC CAG GCG TTC ACC AAG CTG AAC GAG GTA TCC ATT CCG ACG GTG GAA GAA CTG CTG GCG GCA CAT AAT
 A L R C H S Q A F T I K L K K E V S I P T V E E L L A A H N
 901/301
 CCG TGG GCG AAA GTG GTG CCG AAC GAT CGT GAT ATC ACT ATG CGC GAA TTA ACC CCG GCG GTG ACC GGC ACG TTG ACT ACG CCG GTT
 P W A K V V P N D R D I T M R E L T P A A V T G T L T T P V
 991/331
 GGT CGT CTG CGT AAG CTG AAC ATG GCG CCA GAG TTC TTG TCG GCG TTT ACC GTA GGC GAC CAG TTG TTA TGG GGC GCC GCC GAG CCG CTG
 G R L R K L N M G P E F L S A F T V G D Q L L W G A A E P L
 1081/361
 CGT CGA ATG CTG CGC CAG TTG GCG TAG
 R R M L R Q L A *

FIGURE 42. Construction of the DNA vaccine vector pYA3651

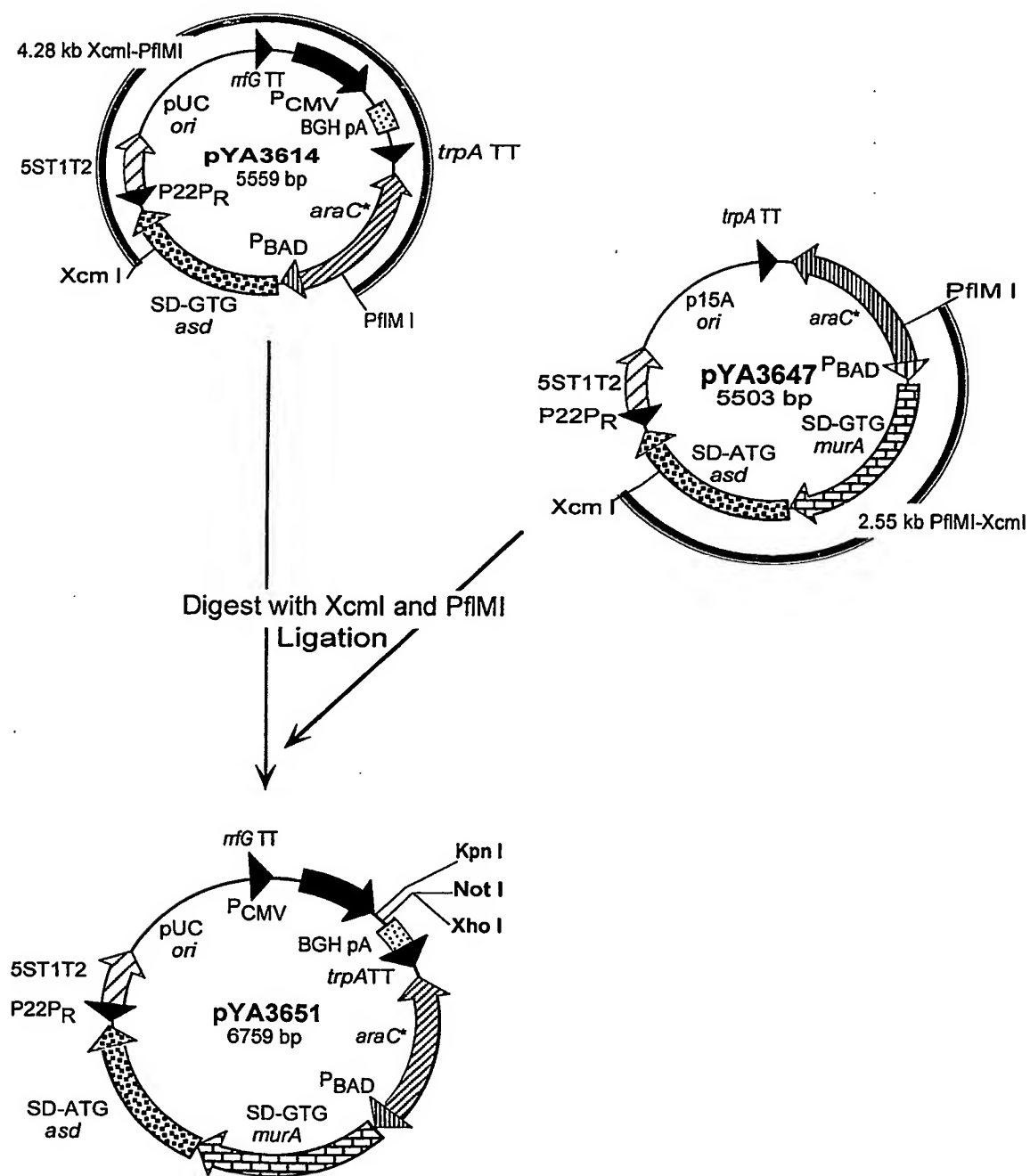


FIGURE 43. DNA sequence of the DNA vaccine vector pYA3651 A. (1-3300 bp)

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1  GACTCTTCGC GATGTACGGG CCAGATATAC GCGTTAACTG CAGTCTAGAT TATGCGAAAG GCCATCTCTG CCGATGGCCT TTTTGTTTAA ACGGATCCGC 100
101 GACATTGAT ATTGACTAGT TATTATATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCGCGGTT ACNTAACTTA CCGTAAATGG 200
201 CCGCGCTGGC TGACCGGCCA ACGACCCCGC CCCATTGACG TCAATTAATGA CGTATGTTC CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG 300
301 GTGGACTATT TACGGTAAAC CAGTACATG ACCTTATGGG ACTTTCCTAT TTGGCAGTAC ATCTAGCTAT TAGTCATCGC TATGACGTCAA TGACGGTAAA TGGCCCGCCT 400
401 GGCATTATGC CCAGTACATG ACCTTATGGG ACTTTCCTAT TTGGCAGTAC ATCTAGCTAT TAGTCATCGC TATGACGTCAA TGACGGTAAA TGGCCCGCCT 500
501 CATCAATGGG CGTGGATAGC GGTTCGATGC CCGGATGTTT CCAAGTCTCC ACCCATTTG CGTCAATGGG AGTTTGTGTTT GGCACCAAAA TCAACGGGAC 600
601 TTTCCTAAAT GTCTGTAACA CTCGCCCCCA TTGACGCAAA TGGGCGGTAG CCGGTACGGG TGGGAGGTCT ATATAAGCAG AGCTCTCTGG CTAACCTAGAG 700
701 AACCCACTGC TTACTGGCTT ATCGAAATTA ATACGACTCA CTATAGGAG ACCCAAGCTG GCTAGCGTTT AAACCTAAGC TTGTACCCGA GCTCGGATCC 800
801 ACTAGTCCAG TGTGTGGGAA TTCTGCAGAT ATCCAGCACA GTGGCGGCGG CTCGAGTCTA GAGGCGCGCT TTAACCCGCG TGATCAGCCT CGACTGTGCC 900
901 TTCTAGTTGC CAGCATCTG TTGTTTGCCC CTCCGCCGTG CCTTCTTGA CCTTGAAGG TGCCACTCCC ACTGTCTCTT CCTAATAAAA TGAGGAAATT 1000
1001 GATCGCATTT GTCTGAGTAG GTGTCTGCTT TCTACTGGGC GGTTCATGCT ATCTGGGG GTGGGTGGG ACAGCACAGC AAGCGGAGG AATGGGAAGA CAATAGCAGG CATGCTGGG 1100
1101 ATGCGTGGG CTCTATGGCT TCTATGGGCT TCTACTGGGC GGTTCATGCT ATCTGGGG GTGGGTGGG ACAGCACAGC AAGCGGAGG AATGGGAAGA CAATAGCAGG CATGCTGGG 1200
1201 CACCTGAAGT CAGCCCATTA CGATATAAGT TGTTCGAAAG TCTAGCCCGC CTAATGAGCG GGTCTTTT TTAATGCGAA TTTCAGTGCA ATTTATCTCT TCAATATGTG 1300
1301 CTGTCAATG GACGAGCAG GGAATCTGCA AACCTATGC TACTCGTCA AGCGTCAAT TGTCTGATTC TTAATGAGT GGTACCAATT ATGACCACTT GACGGCTACA 1400
1401 TCATTACATT TTCTCTACA ACCGACAGA AACTCGCTCG GGTGCGCCC CAAAGCAGC TTGCGCTGAC TAAATGCGTG GTCTCGCG CAGCTTAAGA CGTAAATCCC 1500
1501 TAATTGGACC GACGTGGCG ATAGCATCC GTGACAGAC CGACGGCGAT TCCATCGGTG GATGAGCGA CTCGTTAATC GCTTCCATGC GCCGAGTAA CAATTGCTCA AGCAGATTTA 1600
1601 ATGTACTGAC AAGCTCGCG TACCGGATTA TCCATCGGTG GATGAGCGA CTCGTTAATC GCTTCCATGC GCCGAGTAA CAATTGCTCA AGCAGATTTA 1700
1701 TCGCCAGCAG CTCGGAATAG CGCCCTTCCC GTTAAATGAT TGCCCAACA GGTGCTGAA ATGCGGCTGG TGCGCTTCAT CCGGCGGAAA 1800
1801 GARACCCGTA TTGGCAATA TTGACGGCCA GTTAAAGCCAT TCATGCCAGT AGGCGCGCGG ACGAAAGTAA ACCACTGGT GATACCACTC GCGAGCCTCC 2000
2001 GGATGACGAC CGTAGTGATG AATCTCTCTT GCGCGGAA CA GCAAAATATC ACCCGTGG CAGACAAATT CTGCTCCCTG ATTTTTCACC ACCCCTGAC 2100
2101 CCGNATGGT GAGATTGAGA ATATAACCTT TCATCCCAG CCGTGGGTG ATAAAAAAT CAGATAAACC GTTGGCCTCA ATGCGGCTTA AACCGCCAC 2200
2201 CAGATGGGG TTAAAGAGT ATCCGGGCG CAGGGATCA TTTTGGGCTT CAGCCATATC CAGACATATC CCAACATTA GAGAGAGAAC CAATTGTCCA 2300
2301 TATGTGATCA GACATTGCGG TCACTGCGTC TTTTACTGGC TCTTCTGCTT AACCAACCG GTAAACCCCG TTATTAAGA CATTTCTGTA AAGAGCGGA 2400
2401 CCAAGCCAT GACAAAACG CGTAACAAA GTGTCTATA TCAGGGCAGA AAGTCCACA TCTCTACTGT TTTCTCAVAC CCGTTTTTTT GGGCTAGCGA ATCTGAGAA 2500
2501 ATTTTATCC ATAGATTAG CCGATCCTAC CTGACGCTTT TTATCGCAAC GATCTACAA GAAGTACAA TTTCCGGCGC TAAATAAGT TACTTCTATC TCCTTTTTC 2600
2601 CAAATAAGT GATATAATT CGTGTTCAGG GGCACACGAA GCTCCAGG CCGAAACGTC CCGAACCTGA AAGACGTGGA TACATCATAG AAGTGTCTAA GCGAGCTGG 2700
2701 CGCATACTG GCGGAAGAAC CCGTAGAGAT CCGAAGCTTC CCGAAGCTTC ACCTTATGCT TGTGAGTAC GATCTGGTAA AACTATGGG TGTCTTATC TGGGCGCTGG 2800
2801 GAACGTAAT GTTCTGTGCA TATTGATGCC CCGCAAGTTA ATGATTTCTG TGTGAGTAC GATCTGGTAA AACTATGGG TGTCTTATC TGGGCGCTGG 2900
2901 GACGCTGCT AGCGGCTTT GGTCAAGGGC AACTTTTCACT ACCTTATGCT TGTGAGTAC GATCTGGTAA AACTATGGG TGTCTTATC TGGGCGCTGG 3000
3001 ATTAGCGCG ACCATCAAC TGAAGAAGG TTAGGTTAAA GCTTCCGCTG ATGCTGCTTT GAAAGTCTGA CATATCTGGA TGTATTAAGT CAGCGTTGCG 3100
3101 GCAACGGTGA CCATCATGTG TGTGCAACC CTGCGGGAAG GCACACCAT TATTGAAC GACGCGCTG AACCGGAAT CBTCTGATAC GCGAATCTCC 3200
3201 TGATTACGCT GGGTGGGAAA ATTAGCGGTC AAGGCACCGA TCGTATCTGC ATCGAAGTGT TGGAACTTT AGCGGCGCGT GTCTATCGCG TTCTGCCCGA 3300

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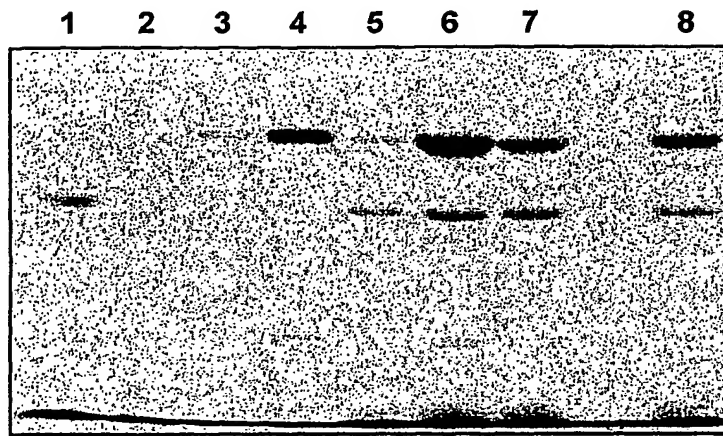
FIGURE 43. (continued) DNA sequence of the DNA vaccine vector pYA3651 B. (3301-6759bp)

3301 TCCTATCGAA ACCGGTACTT TCCTGGTGGC GGGGGCGATT TCTCGGGGCA AATTATCTG CCGTAACGGC CAGCCAGATA CTCTGACGC CGTCTGGCG 3400
 3401 AAATCGGTG AGCTGGAGC GGACATCGAA GTCCGGCGAAG CTGTGATATG CATGCCAAAC GTCCGAAGG TGTAAAGTA GTTACCGGC 3500
 3501 CGCATCCGC ATTCCCGACC GATATGCAGG CCAGTTTAC GCTGTGTAAC CTGTGGCAG AAGGACCCG GTTTATCACC GAAACGTCT TTGAAAACCG 3600
 3601 CTTTATGCAT GTGCCAGC TGAGCCGTAT GGGCGCGCAG GCCCAATCG AAGCAATAC CGTTATTGT CACGGTGTG AAAAATTTT TGGCGCACAG 3700
 3701 GTTATGGCAA CCGATCTGC TGCATCAGCA AGCTGTGTG TGGCTGGCTG TATTGCGGA GGGACGACG TGGTTCATC ATCGATCGTG 3800
 3801 GCTACGAAC CATTAAGAC AAATCGCGC AATGGTCGG CTTTGGTCTA TGCAACGCAT GTTAGAGGAG CGGATTTTC CCCTGTTTC TTTTCTACCT 3900
 3901 GGTATTATCG GCTGGCGCG AATGGTCGG CTTTGGTCTA TGCAACGCAT GTTAGAGGAG CGGATTTTC CCCTGTTTC TTTTCTACCT 4000
 4001 CCCAGTTGG ACAGGCGCG CCCACCTTC GGCACCTC CACCGGCAG CTACAGGAGC CTTTGGTCTA GGTAGGCTG ATATCATCGT 4100
 4101 GACCTGCCAG GGGCGGAT ATACCAACA AATTATCCA AAGCTGCGG AAGCGGATG GCAGGTTAC TGGATTGAT GGTCTTCTAC GCTGCGCATG 4200
 4201 AAAGATGAT CCATTATT TCTCGACCG GTCAACCCAG AGCTGATTAC CAGCGGCTG AACATGGCG TGAAGACCTT TGTGGCGGT AACTGTACC 4300
 4301 TTAGCTGAT GTTGATGTC GTGGGCGTC TCTTGGCCA TAATCTCGT GACTGGGTAT CCGTCGCGC CTATCAGGC GCCTCCGGC GCGGCGCGC 4400
 4401 CCATATGCG GAGCTGTAA CCCAGATGG TCAGTTGAT GGCATGTCG CCATGAACT GGCACGCGC TCTTCGCAA TTCTTGATAT TGAACGCAA 4500
 4501 GTTACGGCAT TGACCGCAG CGGCGAGCT GCGGTTGATA ACTTGGCGT ACCGCTGGG GGAAGCTGA TCCCTGGAT CGACAACAG CTCGATAACG 4600
 4601 GCGAGAGCG CGAAGATGG AAGGCCAGG CGGAACCAA CAAATCTC AATCTGCTT CTTGTGATG GTTGTGTC GCGTCCGGC 4700
 4701 GCTGGCTGT CACAGCAGG CGTTCACAT CAAGCTGAA AAGAGGTAT CCATTCGAC GGTGGAAGA CTGCTGGCG CACATAATC GTGGCGAAA 4800
 4801 GTGTGCGCA ACGATCGTA TATCACTATG CCGGAATTA CCGCGCGC GGTGACCGG ACCTGACTA CGCCGGTGG TCGTCTGCGT AAGCTGAACA 4900
 4901 TGGGCGCAGA GTTCTGTG CCGTTTACCG TAGGCGACCA GTTGTATGG GCGCGCGCG AGCCGCTGG TCGAATGCT CGCCAGTTG CGTAGTCTAG 5000
 5001 CTCACGATA CCGTCGACT GTACATGAC TCGCTCGAA ATTAAGAAC ACTTAATA TCTACTAAG GAATCTTAG TCAAGTTAT TTAAGATGAC 5100
 5101 TTAACATGA ATACAAAT TTACAGCTGAT CAGTATGAT TCCATTATG TCCATTATG TCCATTATG CAGGTTAT CAGGTTAT GTCTCATGAG 5200
 5201 AGAAGATTA TACAGATTAA ATCAGAACG AGAAGCGTC AGAAGCGTC AGAAGCGTC AGAAGCGTC CCGGTTGTC CCGGTTGTC CACCTGACCC 5300
 5301 CATGCCGAC TCAGAGTGA AAGCCGTAG CCGCGATGT TCGGTTGTT TCGGTTGTT TCGGTTGTT TCGGTTGTT TCGGTTGTT TCGGTTGTT CAGTGAAGG 5400
 5401 TCACTCGAA GACTGGCCT TCGGTTGTT TCGGTTGTT TCGGTTGTT TCGGTTGTT TCGGTTGTT TCGGTTGTT TCGGTTGTT TCGGTTGTT CAGTGAAGG 5500
 5501 CAGCGCCCG GAGGTGGC GGCAGGACG CCGCCATAA CCGCCATAA CCGCCATAA CCGCCATAA CCGCCATAA CCGCCATAA CCGCCATAA CCGCCATAA 5600
 5601 AAATCTTTT TGTATTAT TCTAAATATG TATCCGCTCA TATCCGCTCA TATCCGCTCA TATCCGCTCA TATCCGCTCA TATCCGCTCA TATCCGCTCA 5700
 5701 CAGAGGTAA CAGAACGTC GGTGATCG GGTGATCG GGTGATCG GGTGATCG GGTGATCG GGTGATCG GGTGATCG GGTGATCG GGTGATCG 5800
 5801 GGCAGCCCTG AACAGTCAC GGTGATCG GGTGATCG GGTGATCG GGTGATCG GGTGATCG GGTGATCG GGTGATCG GGTGATCG GGTGATCG 5900
 5901 TTTTCTTAA TACATTCAA TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG 6000
 6001 GATCTAGT GAAGATCTT TTTGATAATC TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG 6100
 6101 ATCTCTTGA GATCTCTT TTTGATAATC TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG TATGATCG 6200
 6201 ACTCTTTTC CAGAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAATATG TACCAATATG TACCAATATG TACCAATATG TACCAATATG TACCAATATG 6300
 6301 CACCGCTTAC ATACTCTGT TGTCTAATCC TGTCTAATCC TGTCTAATCC TGTCTAATCC TGTCTAATCC TGTCTAATCC TGTCTAATCC TGTCTAATCC 6400
 6401 GGATAAGCG CAGCGTCCG CTGTGATCG CAGCGTCCG CTGTGATCG CAGCGTCCG CTGTGATCG CAGCGTCCG CTGTGATCG CAGCGTCCG CTGTGATCG 6500
 6501 TGAGAAAGCG CACGCTTCC CAGAGGAGA AAGCGGAGA GGTATCCGT AAGCGGAGA GGTATCCGT AAGCGGAGA GGTATCCGT AAGCGGAGA 6600
 6601 ACGCTGGTA TCTTTATAGT CTTGCGCACCT CTGATGATG CTTGCGCACCT CTGATGATG CTTGCGCACCT CTGATGATG CTTGCGCACCT 6700
 6701 CAGCAACCG GCCTTTTTC GGTTCCTGCG CTTTGTGCTG CTTTGTGCTG CTTTGTGCTG CTTTGTGCTG CTTTGTGCTG CTTTGTGCTG CTTTGTGCTG 6759

FIGURE 44. DNA and amino acid sequences of ATG-asd gene of pYA3651

1/1
 ATG AAA AAT GTT GGT TTT ATC GGC TGG CGC GGA ATG GTC GGC TCT GTT CTC ATG CAA CGC ATG GTA GAG GAG CGC GAT TTC GAC GCT ATT
 M K N V G F I G W R G M V G S V L M Q R M V E E R D F D A I
 31/21
 91/31
 CGC CCT GTT TTC TTT TCT ACC TCC CAG TTT GGA CAG GCG GCG CCC ACC TTC GGC GAC ACC TCC ACC GGC ACG CTA CAG GAC GCT TTT GAT
 R P V F F S T S Q F G Q A A P T F G D T S T G T L Q D A F D
 151/51
 181/61
 CTG GAT GCG CTA AAA GCG CTC GAT ATC ATC ACC TGC CAG GGC GAT TAT ACC AAC GAA ATT TAT CCA AAG CTG CGC GAA AGC GGA
 L D A L K A L D I I V T C Q G G D Y T N E I Y P K L R E S G
 241/81
 271/91
 TGG CAG GGT TAC TGG ATT GAT GCG GCT TCT ACG CTG CGC ATG AAA GAT GAT GGC ATT ATT ATT CTC GAC CCG GTC AAC CAG GAC GTG ATT
 W Q G Y W I D A A S T L R M K D D A I I L D P V N Q D V I
 331/111
 361/121
 ACC GAC GGC CTG AAC AAT GGC GTG AAG ACC TTT GTG GGC GGT AAC TGT ACC GTT AGC CTG ATG TTG ATG TCG CTG GGC GGT CTC TTT GCC
 T D G L N N G V K T F V G G N C T V S L M L M S L G G L F A
 421/141
 451/151
 CAT AAT CTC GTT GAC TGG GTA TCC GTC GCG ACC TAT CAG GCC GCC TCC GGC GGC GGC CAT ATG CGC GAG CTG TTA ACC CAG ATG
 H N L V D W V S V A T Y Q A A S G G A R H M R E L L T Q M
 511/171
 541/181
 GGT CAG TTG TAT GGC CAT GTC GGC GAT GAA CTG GCG ACG CCG TCT TCC GCA ATT CTT GAT ATT GAA CGC AAA GTT ACG GCA TTG ACC CGC
 G Q L Y G H V A D E L A T P S S A I L D I E R K V T A L T R
 601/201
 631/211
 AGC GGC GAG CTG CCG GTT GAT AAC TTT GGC GTA CCG CTG GCG GGA AGC CTG ATC CCC TGG ATC GAC AAA CAG CTC GAT AAC GGC CAG AGC
 S G E L P V D N F G V P L A G S L I P W I D K Q L D N G Q S
 691/231
 721/241
 CGC GAA GAG TGG AAA GGC CAG GCG GAA ACC AAC AAG ATT CTC AAT ACT GCC TCT GTG ATT CCG GTT GAT GGT TTG TGT GTG CGC GTC GGC
 R E E W K G Q A E T N K I L N T A S V I P V D G L C V R V G
 781/261
 811/271
 GCG CTG CGC TGT CAC AGC CAG GCG TTC ACC ATC AAG CTG AAA GAG GTA TCC ATT CCG ACG GTG GAA GAA CTG CTG GCG GCA CAT AAT
 A L R C H S Q A F T I K L K K E V S I P T V E E L L A A H N
 871/291
 901/301
 CCG TGG GCG AAA GTG GTG CCG AAC GAT CGT GAT ATC ACT ATG CGC GAA TTA ACC CCG GCG GTG ACC GGC ACG TTG ACT ACG CCG GTT
 P W A K V V P N D R D I T M R E L T P A A V T G T L T T P V
 961/321
 991/331
 GGT CGT CTG AAG CTG AAC ATG GCG CCA GAG TTC TTG TCG GCG TTT ACC GTA GGC GAC CAG TTG TTA TGG GGC GCC GCC GAG CCG CTG
 G R L R K L N M G P E F L S A F T V G D Q L L W G A A E P L
 1051/351
 1081/361
 CGT CGA ATG CTG CGC CAG TTG GCG TAG
 R R M L R Q L A *

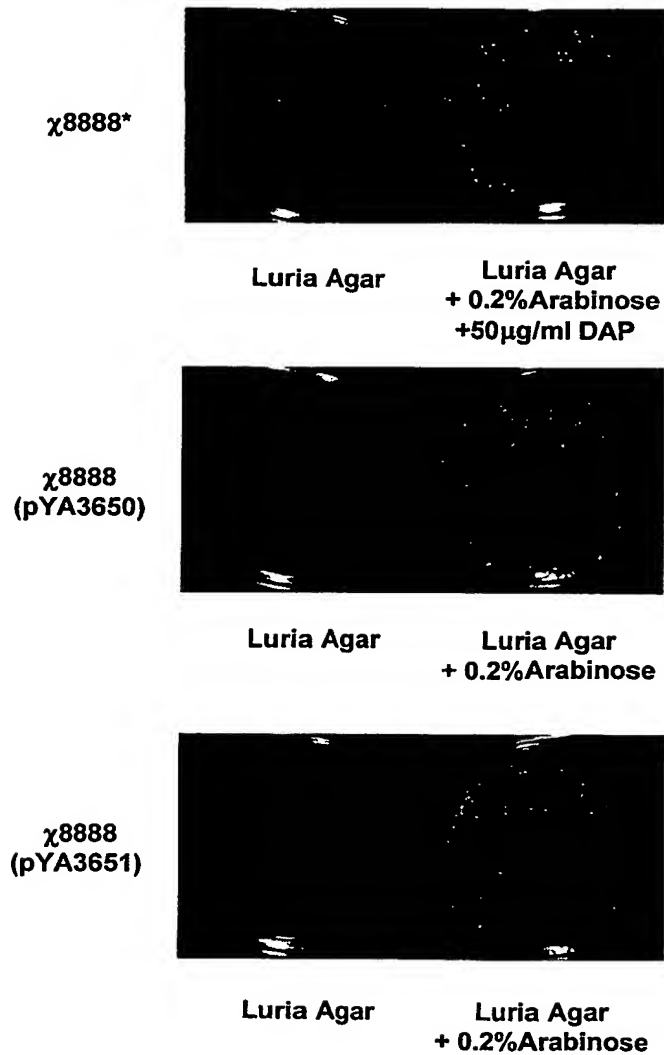
FIGURE 45. Immuno Blot Analysis on AraC P_{BAD} Asd Vectors Using Rabbit Anti-Asd Serum.



Lanes:

1. χ 6212 (*E. coli* Δ asdA4)
2. χ 8276 (*S. typhimurium* UK-1 Δ asdA16)
3. pYA3530 Asd-GTG (Fig. 2A) in χ 8276
4. pYA3450 Asd-ATG in χ 8276
5. pYA3565 Asd-TTG in χ 8276
6. pYA3450 Asd-ATG in χ 6212
7. pYA3530 Asd-GTG in χ 6212
8. pYA3656 Asd-TTG in χ 6212

FIGURE 46. DAP-less and muramic-less death in host strain with DNA vaccine vectors



The results shows phenotypic properties of recombinant host-vector strains displaying arabinose-dependent growth and regulated cell lysis in the absence of arabinose.

$\chi 8888 \Delta asdA::araC P_{BAD} c2, \Delta P_{murA}::araC P_{BAD} murA, \Delta araBAD, \Delta araE, \Delta endA, \Delta gmd-fcl, \Delta relA$

FIGURE 47. Change in body temperature as a consequence of oral immunization of 8-week-old female BALB/c mice with live host-vector systems for delivery of DNA vaccine vectors by regulatable cell lysis in vivo

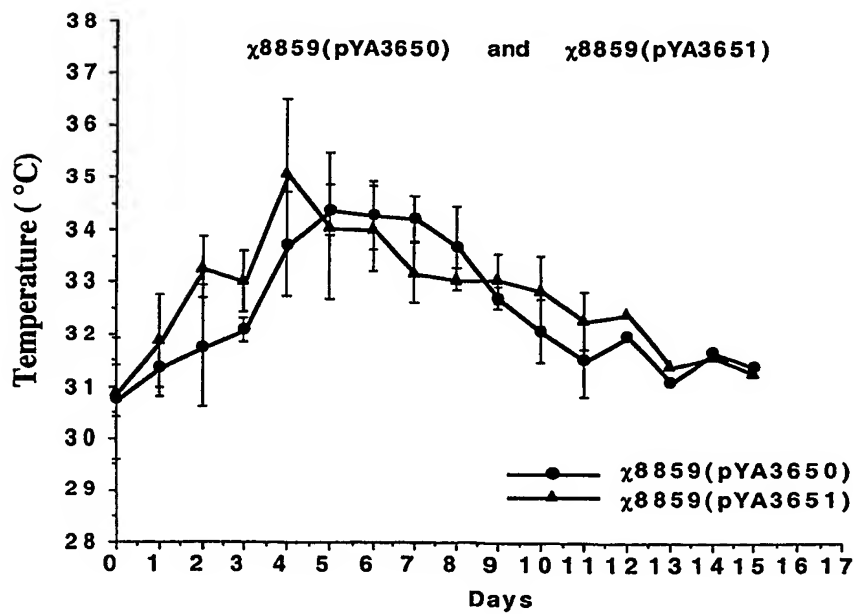
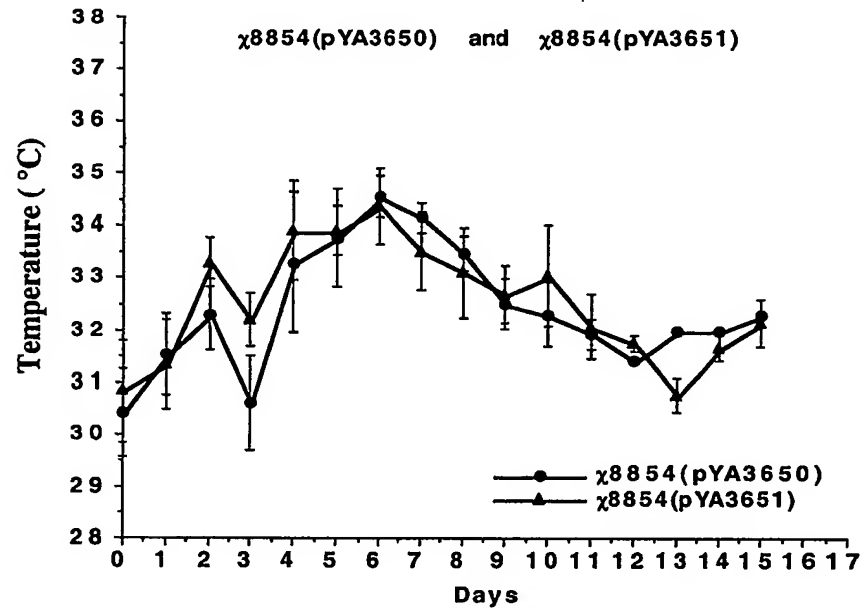
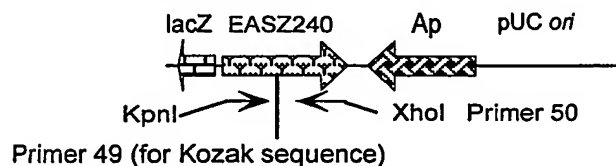
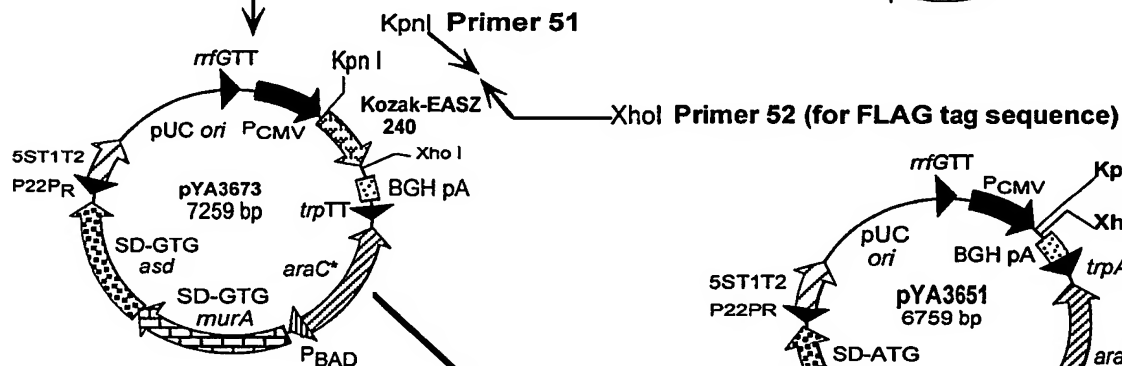


FIGURE 48. Construction of pYA3674 (pYA3650 specifying expression *Eimeria acervulina* EASZ240- FLAG) and pYA3675 (pYA3651 specifying EASZ240-FLAG)

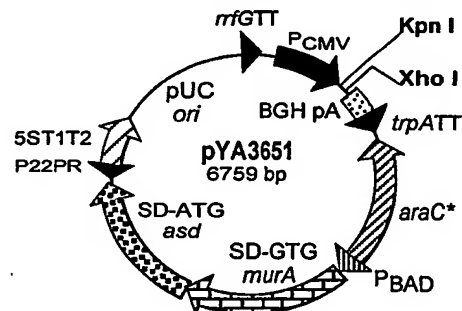
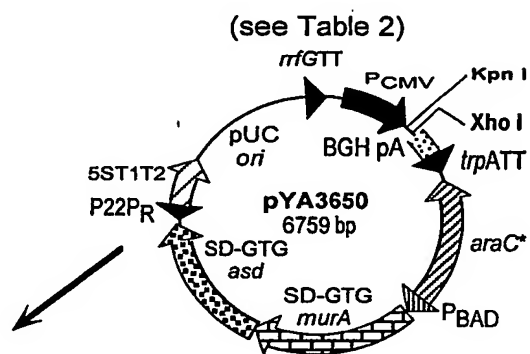
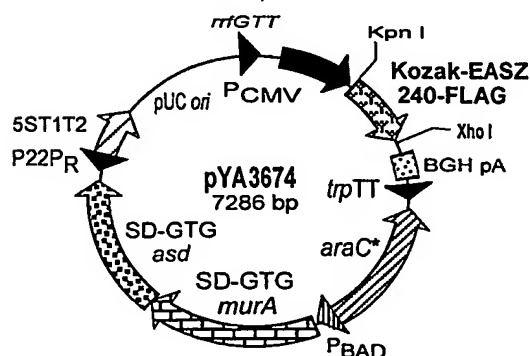
pUC19-EASZ240(#1)



Introduce Kozak sequence and initiation codon (ATG) into EASZ240 by PCR using primer 49 and 50, digest 579 bp PCR product with KpnI and XhoI and insert into KpnI and XhoI of pYA3650



Introduce FLAG tag into c-terminal of EASZ 240 by PCR using Primer 51 and 52, digest 611 bp PCR product with KpnI and XhoI and insert at KpnI and XhoI of pYA3650



Introduce EASZ240-FLAG into pYA3651 at KpnI and XhoI site.

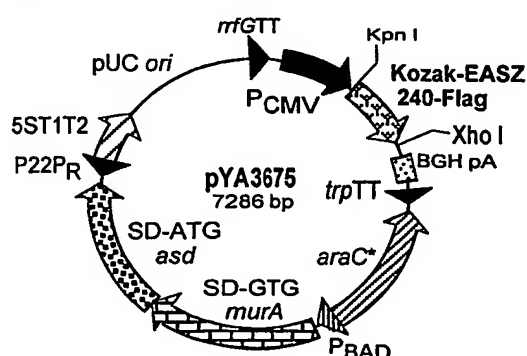


FIGURE 49. Construction of pYA3677 (pYA3650 specifying the expression of the *Eimeria acervulina* EAMZ250-FLAG) and pYA3678 (pYA3651 specifying EAMZ250-FLAG)

pUC19-EAMZ250 (#3)

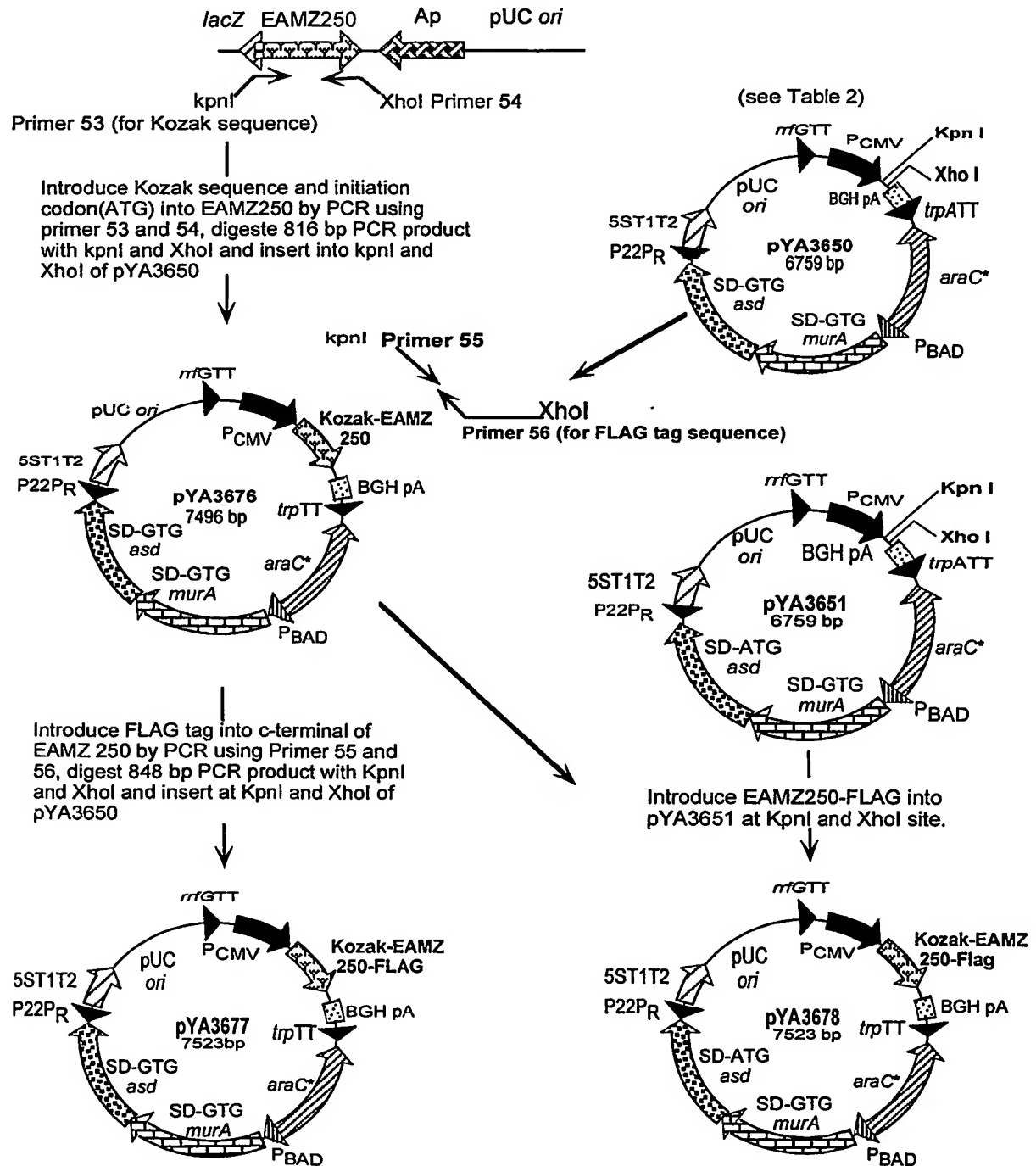


FIGURE 50. DNA and amino acid sequences of EASZ-240 with FLAG fusion in pYA3674 and pYA3675

5' - G GTA CCA GGA GCC GCC ACC ATG GCA-
KpnI SD Kozak start A codon

cgt ttc ttt gta ttt cct tac tca gtt aaa atg ggt gaa gag gct gat act cag gcg tgg gat acc tca gtg aag gaa tgg ctc gtg gat
R F F V F P Y S V K M G E A D T Q A W D T S V K E W L V D
11th a.a of EASZ240 →

acg ggg aag gta tac gcc gcc ggc att gct agc att gca gat ggg tgc cgc ctg ttt ggc gct gca ata gac aat ggg gag gat gcg tgg
T G K V Y A G I A S I A D G C R L F G A A I D N G E D A W

agt cag ttg gtg aag aca gga tat cag att gaa gtg ctt caa gag gac ggc tct tca act caa gag gac tgc gat gaa gcg gaa acc ctg
S Q L V K T G Y Q I E V L Q E D G S S T Q E D C D E A E T L

cgg caa gca att gtt gac gcc gcc ggc cca aac ggt gtt tat att gga gga att aaa tat aaa ctc gca gaa gtt aaa cgt gat ttc acc
R Q A I V D G R A P N G V Y I G G I K Y K L A E V K R D F T

tat aac gac cag aac tac gac gtg gcg att ttg ggg aag aac aag ggt ggc ggc ggt ttc ctg att aag act ccg aac gac aat gtg gtg att
Y N D Q N Y D V A I L G K N K G G G F L I K T P N D N V V I

gct ctt tat gac gag gag aaa gag cag aac aaa gca gat gcg ctg aca acg gca ctt gcc ttc gct gag tac ctg tac cag ggc ggc ttc
A L Y D E E K E Q N K A D A L T T A L A F A E Y L Y Q G G F
→ last a.a of EASZ240

GAT TAT AAA GAT GAT GAT GAT GAT GAT GAT TAA TAA CTC GAG-3'
D Y K D D D D K * * XhoI
FLAG tag stop codon

FIGURE 51. DNA and amino acid sequences of EAMZ-250 with FLAG fusion in pYA3677 and pYA3678

5' - G GTA CCA GGA GCC GCC ACC ATG GCT-
 KpnI SD Kozak start A codon

cct ttg ccc ttt tct cct cct tct aca ccg gtc tct cct cct tct aca ccg gtc tct cct cct tct aca
 P L P F S P P S T P V S P P S T P V S T P P S T
 1th a.a of EAMZ250

cca gtc tct cct tct aca ccg gtt tgc cct cct tct aca ccg gtc tgc cct cct tct aca ccg gtc
 P V S P P S T P V S P P S T P V S T P V S P P S T P V

tgc cct cct tct aca ccg gtc tgc cct cct tct aca ccg gtc tgc cct cct tct aca ccg gtt tca cca
 S P P S T P V S P P S T P V S T P V S P P S T P V S P

cct tct aca ccg gtc tca cca cct tct aca ccg gtt tgc cct cct ggc ggt ggc ggc ggc ggc ggc ggc
 P S T P V S P P S T P V S P P S P A P G A V G V N S S L

tgc caa cgc tca acc tcc gag cac tgg cac ggc tca gtt tcc gtg cag ttt gag cgc tgg cga gat cgc acg cct gcc tct ggc cta cga
 S Q R S T S E H W H A S V S V Q F E R W R D R T P A S G L R

ttc gct cca ctc gcc gaa ggg tgg gca att ctg act gcc gca agt tgt aac ctc cac aac atc agg cag cgc cca gcc tcc tct gct gca
 F A P L A E G W A I L T A A S C N L H N I R Q R P G S A A

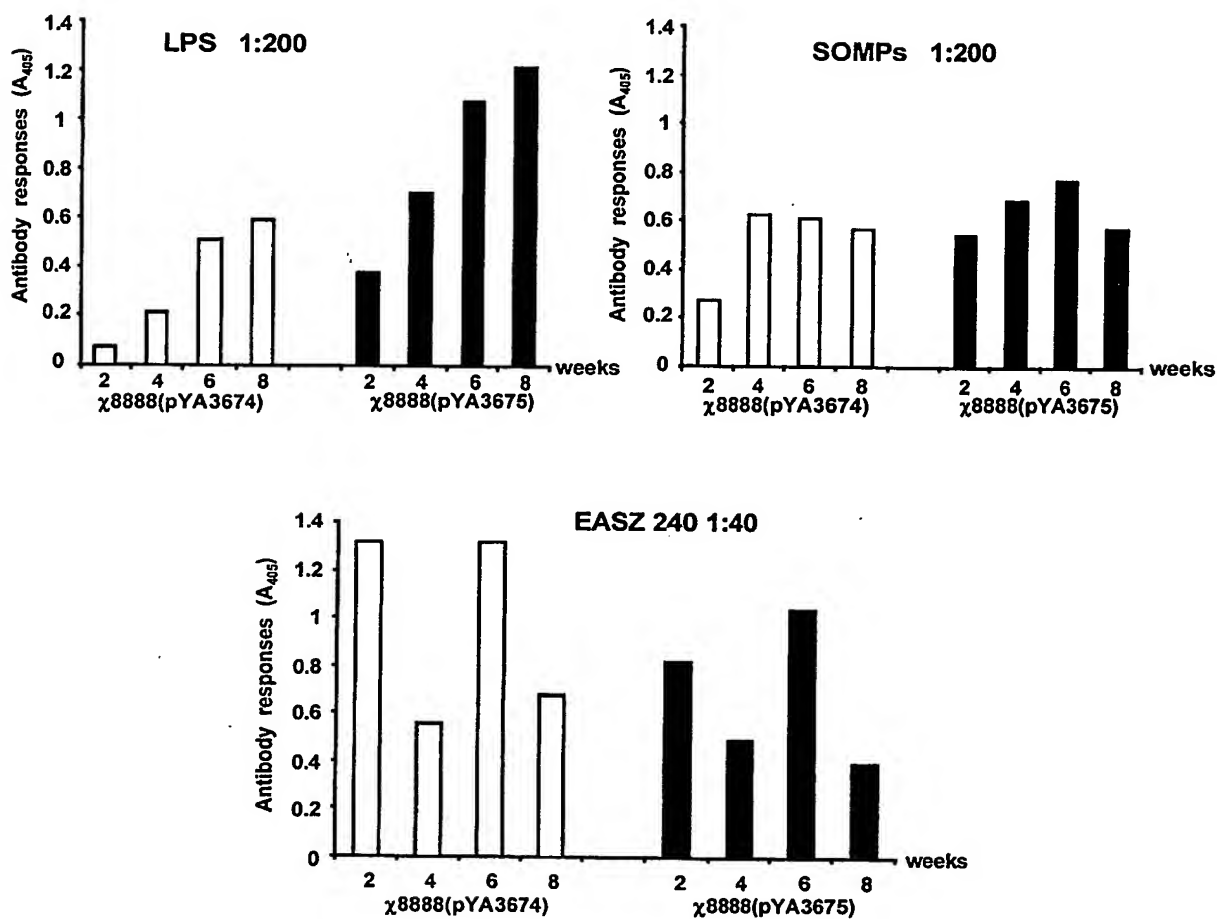
gac cgt cgg cat tgc act cgc tcc act cgc tcc agt cgc cgc atg tgc aga cgt cat cgt cac aag ggc gga ctt cgt ggg ttt gtt tca
 D R R H C T R S T R S S R R M S R R H R H K G G L R G F V S

cga tgc cgt cgg agc gga tgc tgc agg ttc tct tca ttt gct tct ccg acg att cgc tcc aag ctt aca ggt tac ggt gtc gct gac gtc
 R C R R S G C C R F S S F A S P T I R S K L T G Y G V A D V

ggc tgt gga gta ctc ttc gta ctc cgt cac acc gca cgc cgt atc ctc gcg cgt tgc GAT TAT AAA GAT GAT GAT GAT AAA TAA TAA
 G C G V L F V L R H T A R R I L A R S D Y K D D D K * *
 last a.a of EAMZ250 FLAG tag Stop codon

CTC GAG-3'
 XhoI

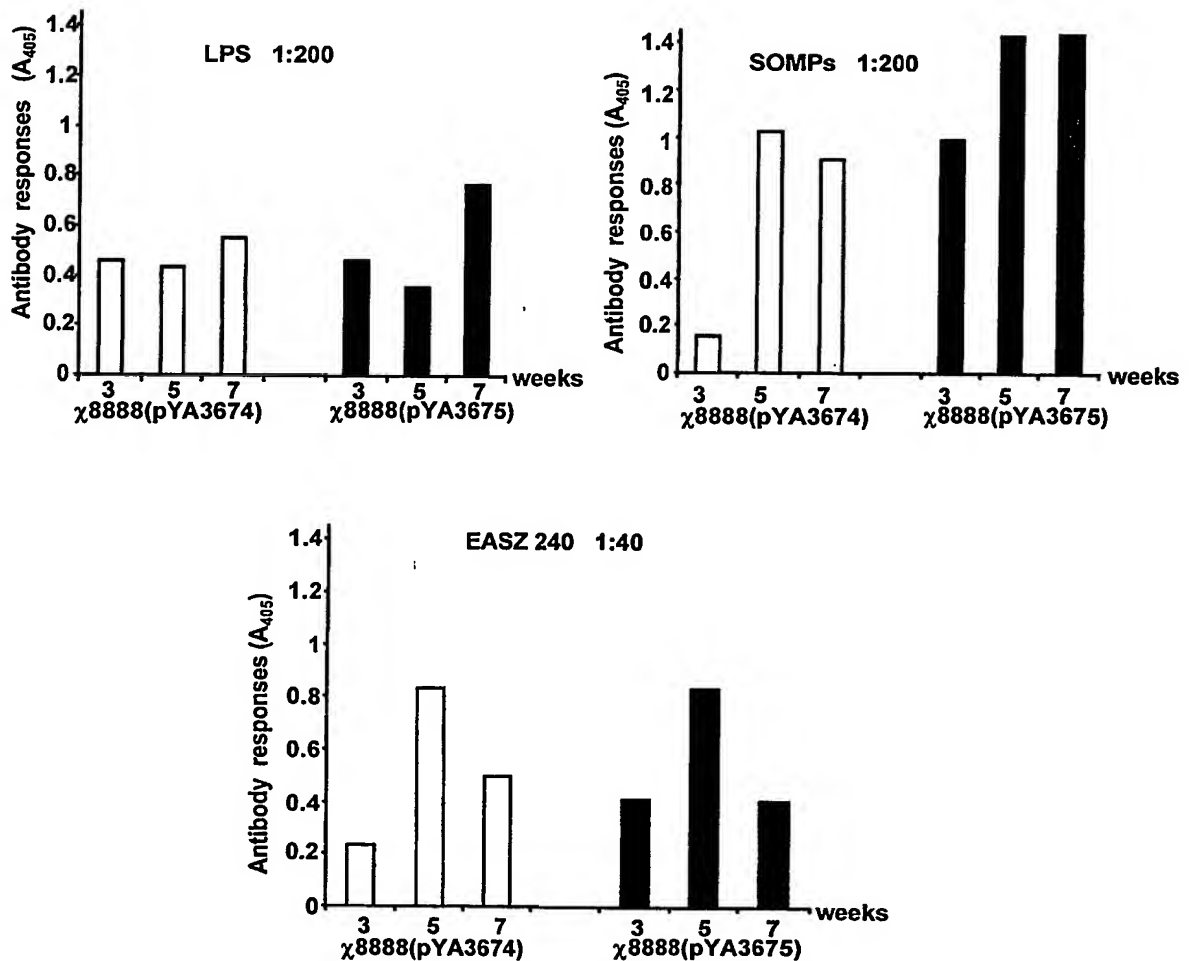
FIGURE 52. Serum of mice IgG responses to *S. typhimurium* LPS, SOMP_s and to EASZ 240.



χ 8888 (pYA3674) with GUG translation start codon of *asd* mRNA induced more rapid cell wall-less death to elicit less of an IgG responses to LPS and SOMP_s but a higher response to EASZ240.

Immunity to *S. typhimurium* is a plus but is not the objective and a very strong induction of immunity to *Salmonella* antigens could compete in induction of desired immune responses to specified protective antigen.

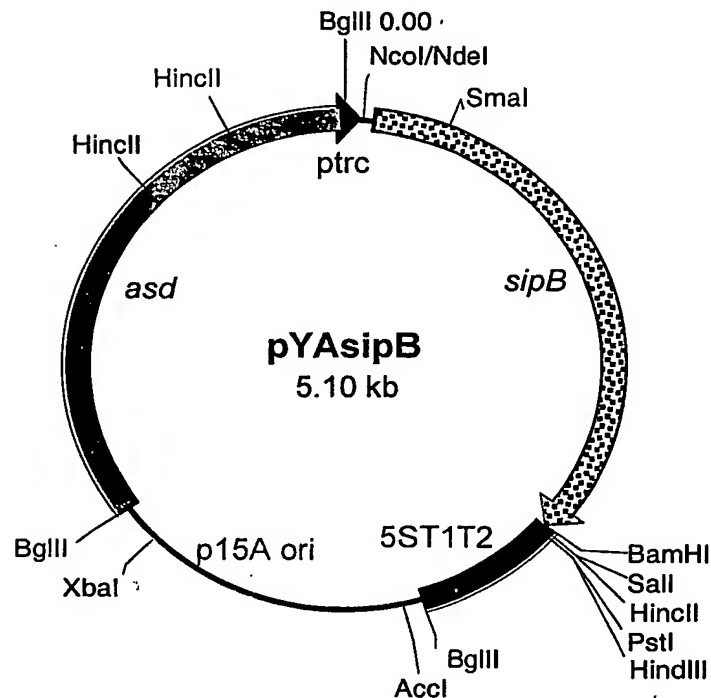
FIGURE 53. Chicken Serum IgG responses to *S. typhimurium* LPS, SOMPs and to EASZ 240



The data represent IgG antibody levels induced in chickens orally immunized with χ 8888 (pYA3674) and χ 8888 (pYA3675) at the indicated weeks after immunization.

Both strains elicited anti-LPS, anti-SOMPs and anti-EASZ 240 IgG.

FIGURE 54. Cloning of *sipB* in Asd vector pYA3332



1818 bp of *sipB* gene (*sipB*-8 to *sipB*+18) was PCR amplified from the *S. typhimurium* UK-1 chromosomal DNA with Primer *sipB*-NdeI and *sipB*-BamHI. This fragment was digested with NdeI enzyme and then filled-in with Klenow enzyme and cut the other end with BamHI. Asd vector pYA3332 was digested with NcoI enzyme and then the overhang removed with a mung bean nuclease and then cut the other end with BamHI enzyme. Then ligate the *sipB* insert with the pYA3332 (blunt ligation).

fused area sequence:

<u>AGGA</u>	AACAGACT	<u>ATG</u>	ACG	CAA
SD		M	T	Q
		<i>sipB</i>		

Primer 57. *sipB*-NdeI: 5'GCAATTCCATATGGTAAATGACGCAAGTAGCATTAG 3'

Primer 58. *sipB*-BamHI: 5'CCGATCCTTTATTTTGGCAGTTTTTATGCG 3'

FIGURE 55. Construction of pYA3681 (pYA3646 with the P_{trc} -MCS TT-pBR *ori* cassette)

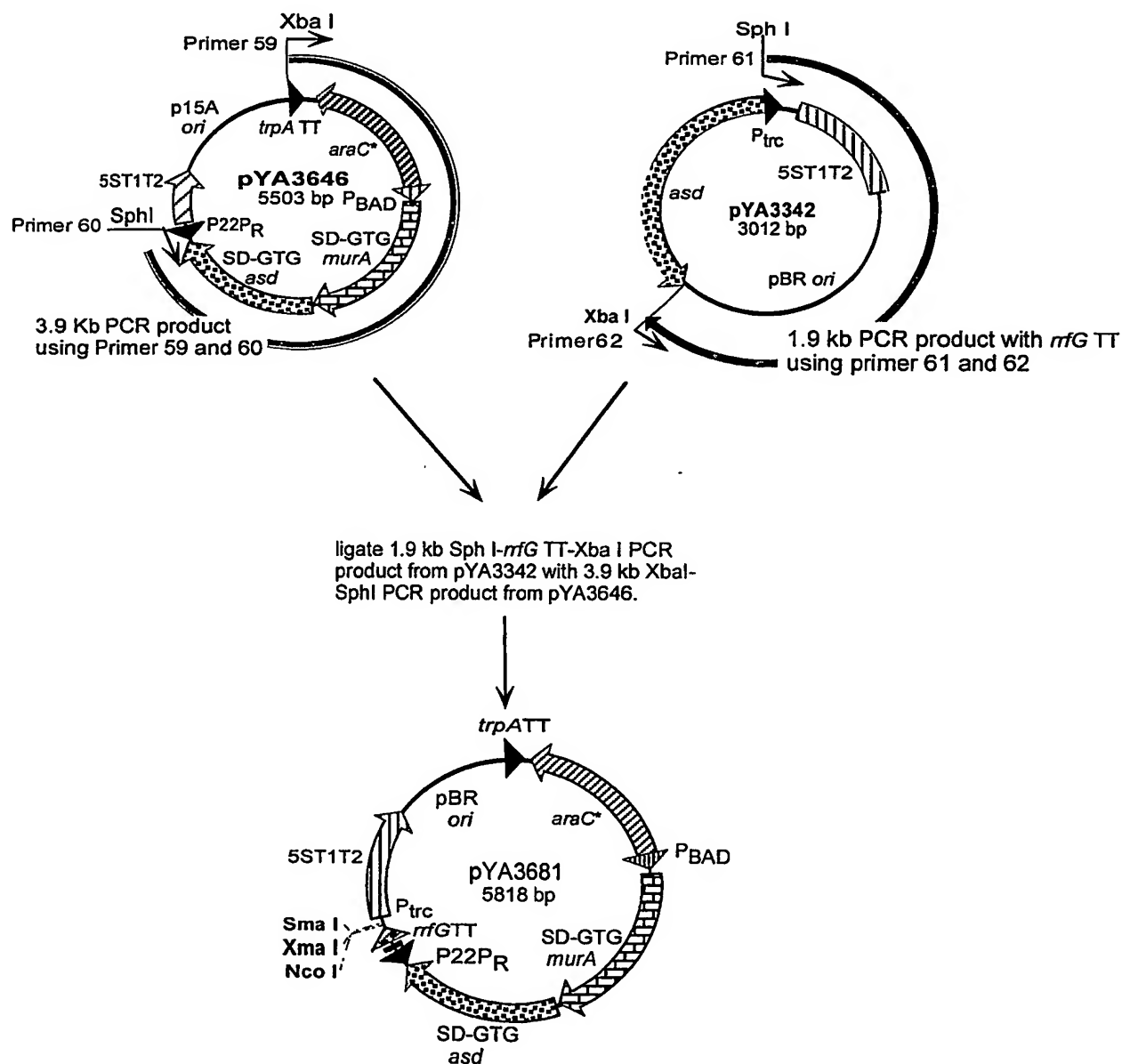
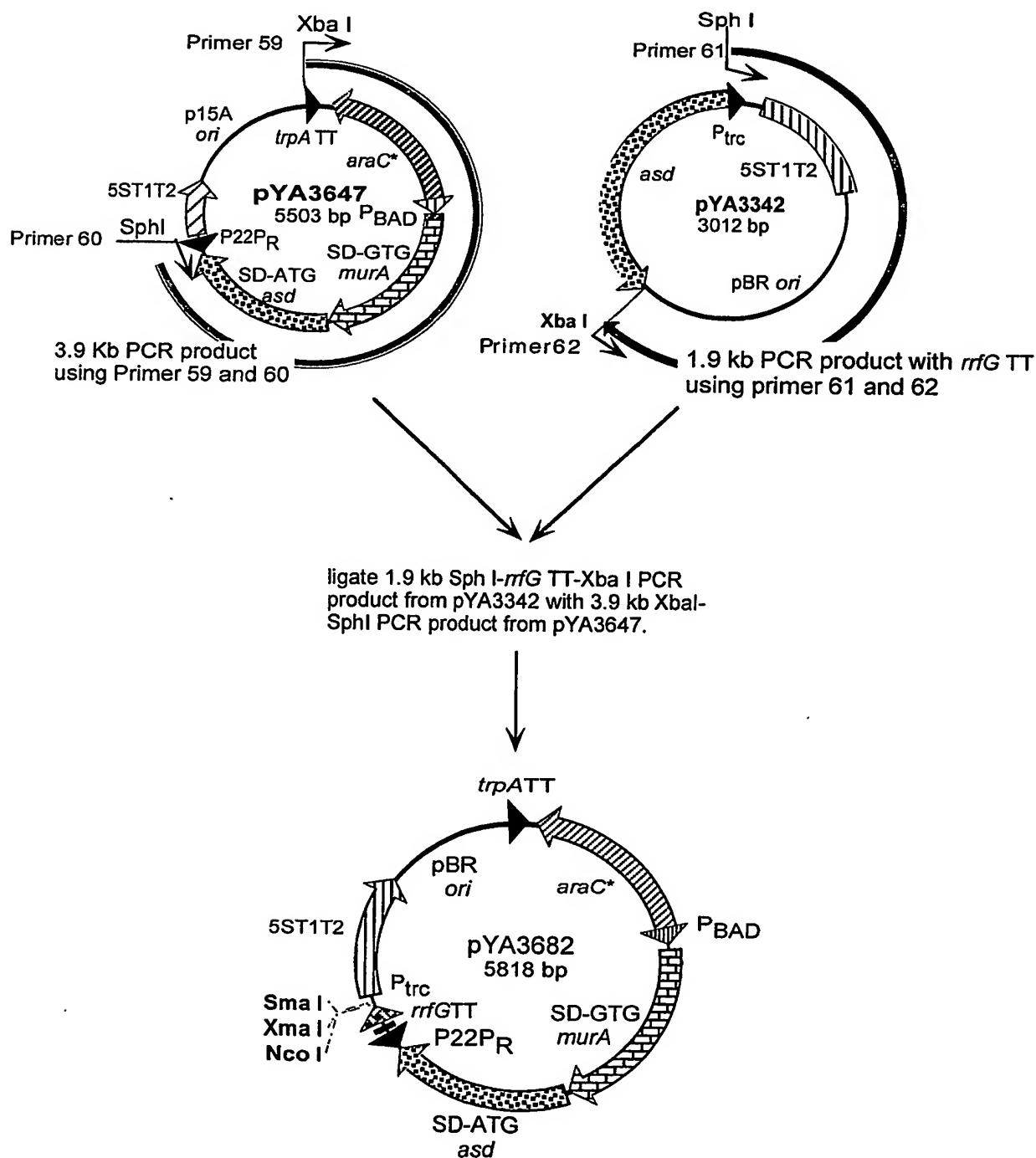


Figure 56. Construction of pYA3682 (pYA3647 with the P_{trc} -MCS TT-pBR *ori* cassette)



The figure shows two circular plasmid maps, pYA3681 and pYA3682, both 5824 bp in size. Both plasmids contain the *trp* operon, including the *trp*ATT promoter, *araC*⁺ gene, *P*_{BAD} promoter, *SD-GTG* (or *SD-ATG* in pYA3682), *murA* gene, *SD-GTG* (or *SD-ATG*), *asd* gene, *P22PR* promoter, *rrfGTT* gene, and *P*_{trc} promoter. The *5S* and *16S* rRNA genes are located at the 5' end, flanked by 5ST1 and T2 sites. Both plasmids have a pBR origin of replication (*pBR ori*) and are digested with *Sma*I, *Xma*I, and *Nco*I. The plasmids are labeled pYA3681 and pYA3682 at the bottom.

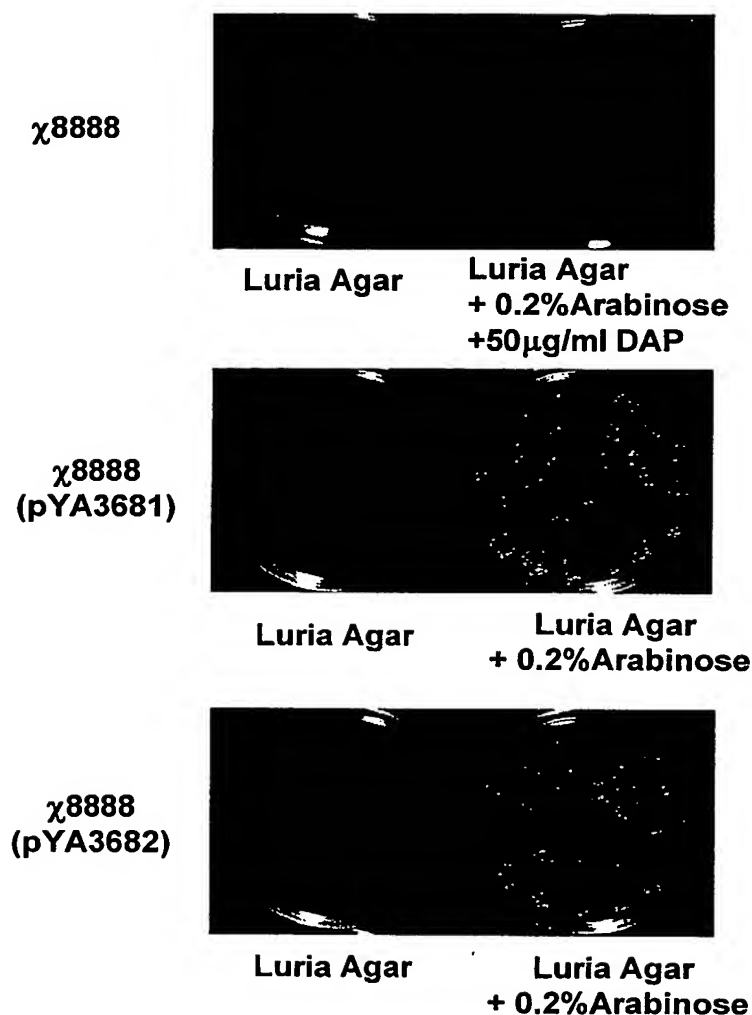
- pBR *ori*.
- P_{trc} promoter for expressing antigen gene.
- araCP*_{BAD} activator-promoter.
- two genes for essential enzymes for synthesis of rigid layer of the bacterial cell wall, *asd* and *murA*.
- GTG start codon for *murA* and either GTG or ATG start codon for *asd* gene.
- P22P_R promoter for synthesis of anti-sense mRNA of *asd* and *murA* genes.
- multiple transcription terminators to block transcription of genes in adjacent segments of vector.

P_{trc} → -35 -10
ATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGT

 SD NcoI
GGAATTGTGAGCGGATAACAATTTACACAGGAACAGACCATGGGA

SmaI/XmaI
TTCGCAATTCCCGGGGATCCGTCGACCTGCAGCCAAGCTCCCAAGCTT

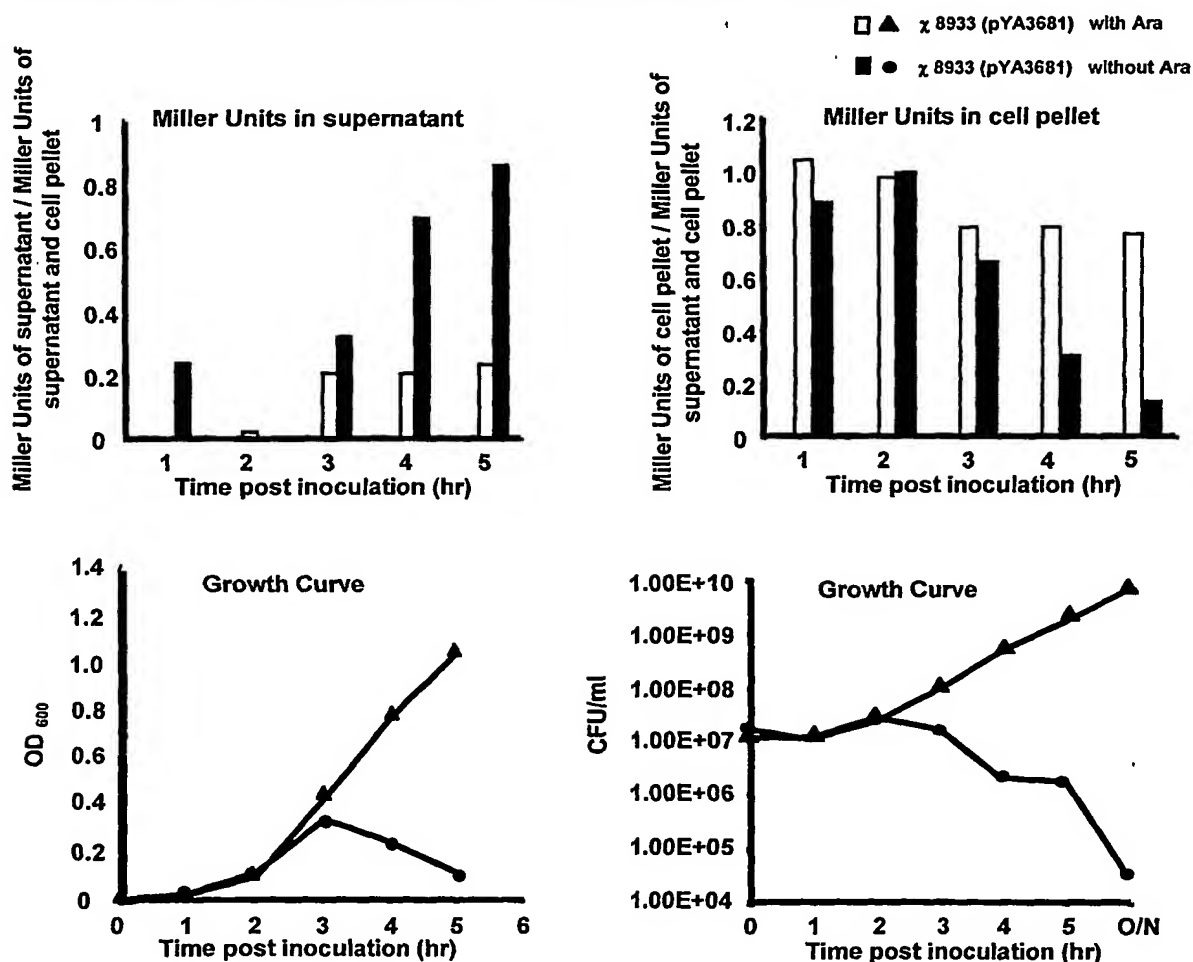
FIGURE 57. DAP-less and Muramic-less Death in host strain with lysis system vectors



The result shows phenotypic properties of recombinant host-vector strains displaying arabinose-dependent growth and regulated cell lysis in the absence of arabinose.

χ8888: $\Delta asdA::araC P_{BAD} c2, \Delta P_{murA}::araC P_{BAD} murA, \Delta araBAD, \Delta araE, \Delta endA, \Delta gmd-fcl$ and $\Delta relA$

FIGURE 58. β -Galactosidase release by cell lysis



β -Galactosidase production from χ 8933 (the *atrB13::MudJ* allele in χ 8888) was used as a cytoplasmic protein marker and as an indicator of cell lysis in the examination of regulated bacterial lysis in vitro.

The ratio of β -Galactosidase activity in supernatant and cell-pellet revealed the extent of cell lysis.

Inoculate (1:400) overnight culture (LB broth with 0.002% Ara) to fresh prewarm LB broth with 0.02% Ara, β -Galactosidase activity in supernatant and cell-pellet were assayed at indicated time point, respectively.

At 3 hr post inoculation (5.4 generations), χ 8933 (pYA3681) growing in LB broth without Ara shows significant cell lysis and β -Galactosidase activity of supernatant.

χ 8933- *atrB13::MudJ* allele in χ 8888

FIGURE 59. Construction of pYA3712 (pYA3681 specifying the expression of codon-optimized rPspA-RX*1)

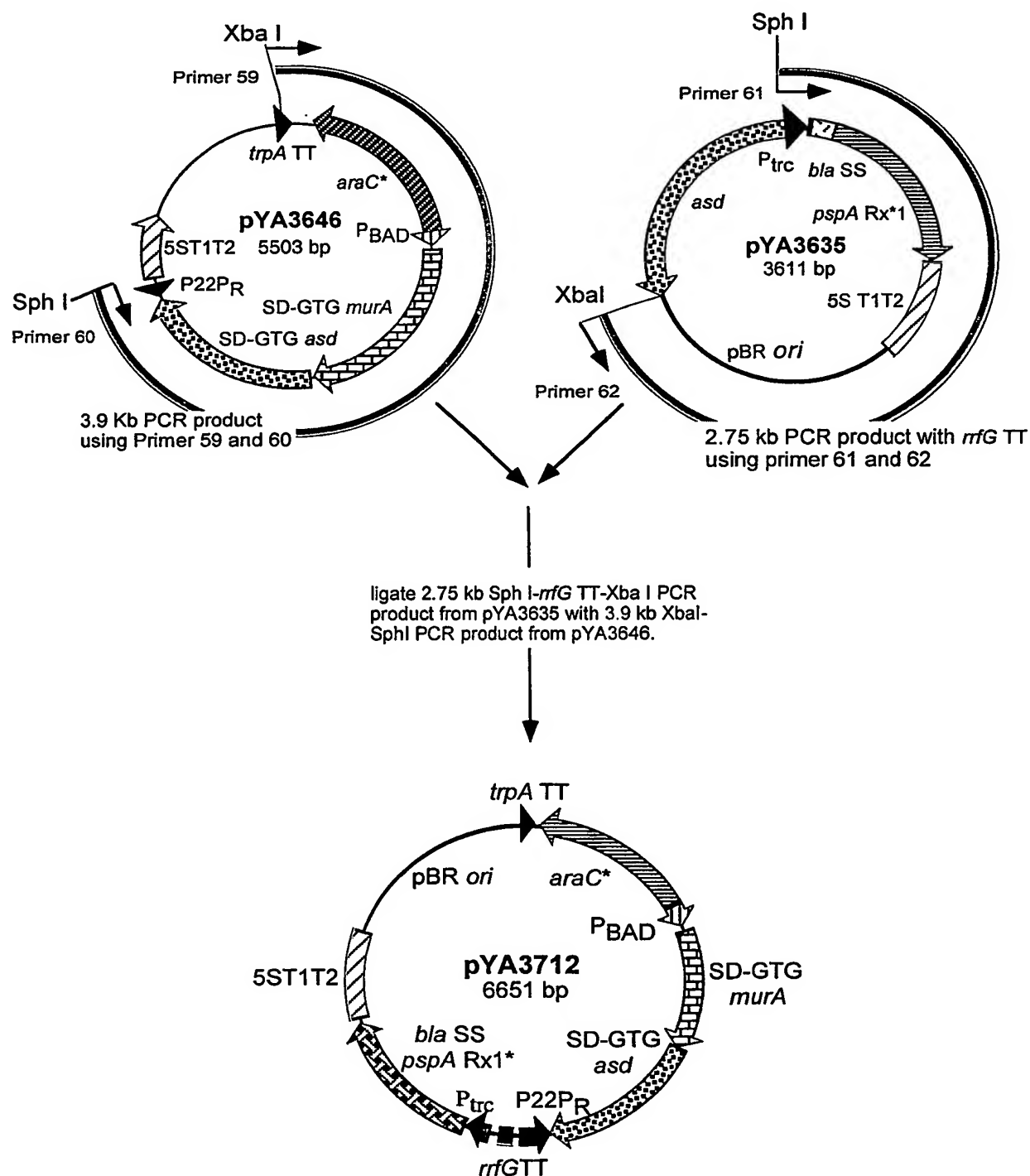


FIGURE 60. Construction of pYA3713 (pYA3682 specifying the expression of rPspA-RX1)

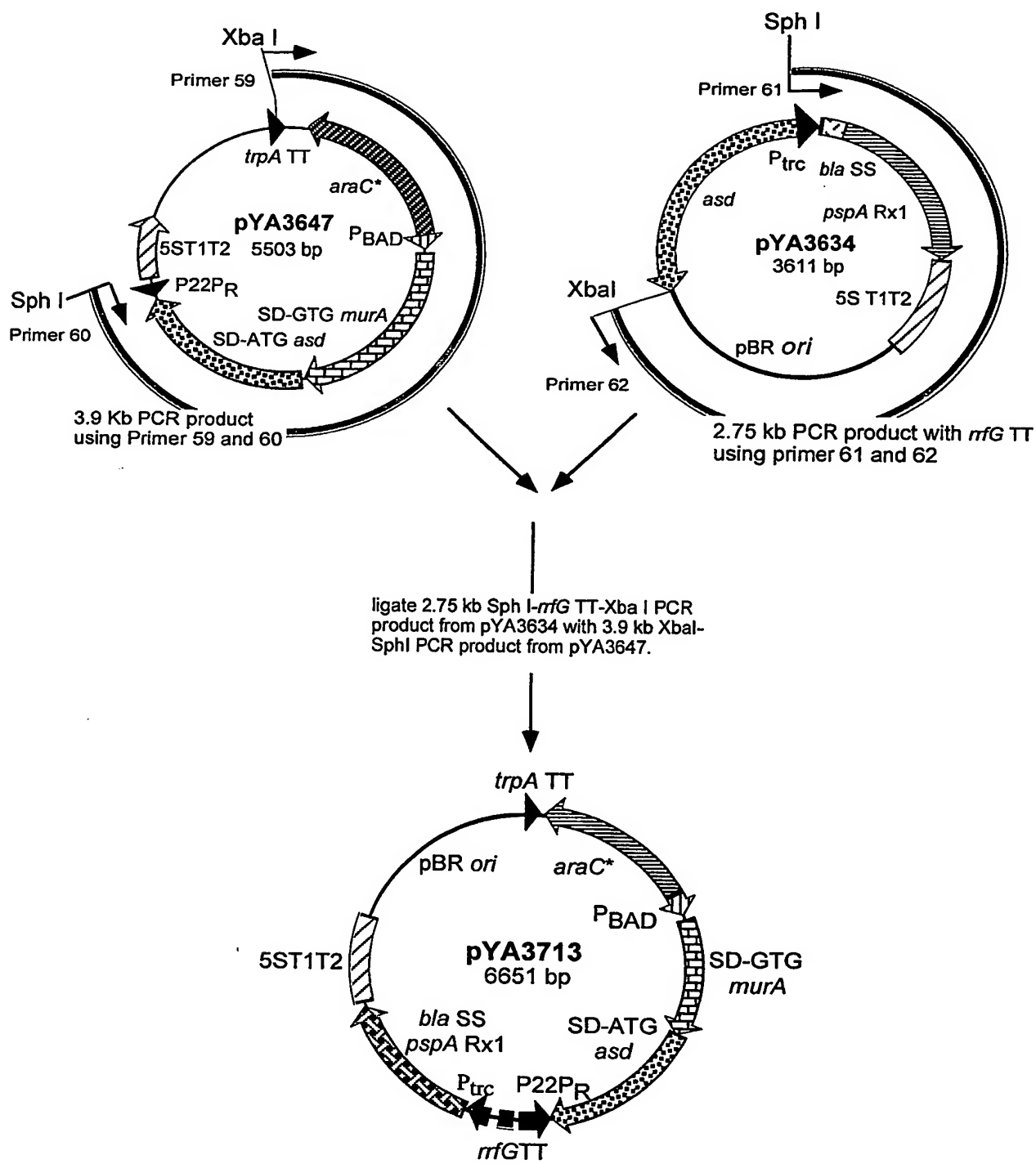


FIGURE 61. Construction of the pYA3681 expressing the HBV core PreS1,S2 sequences.

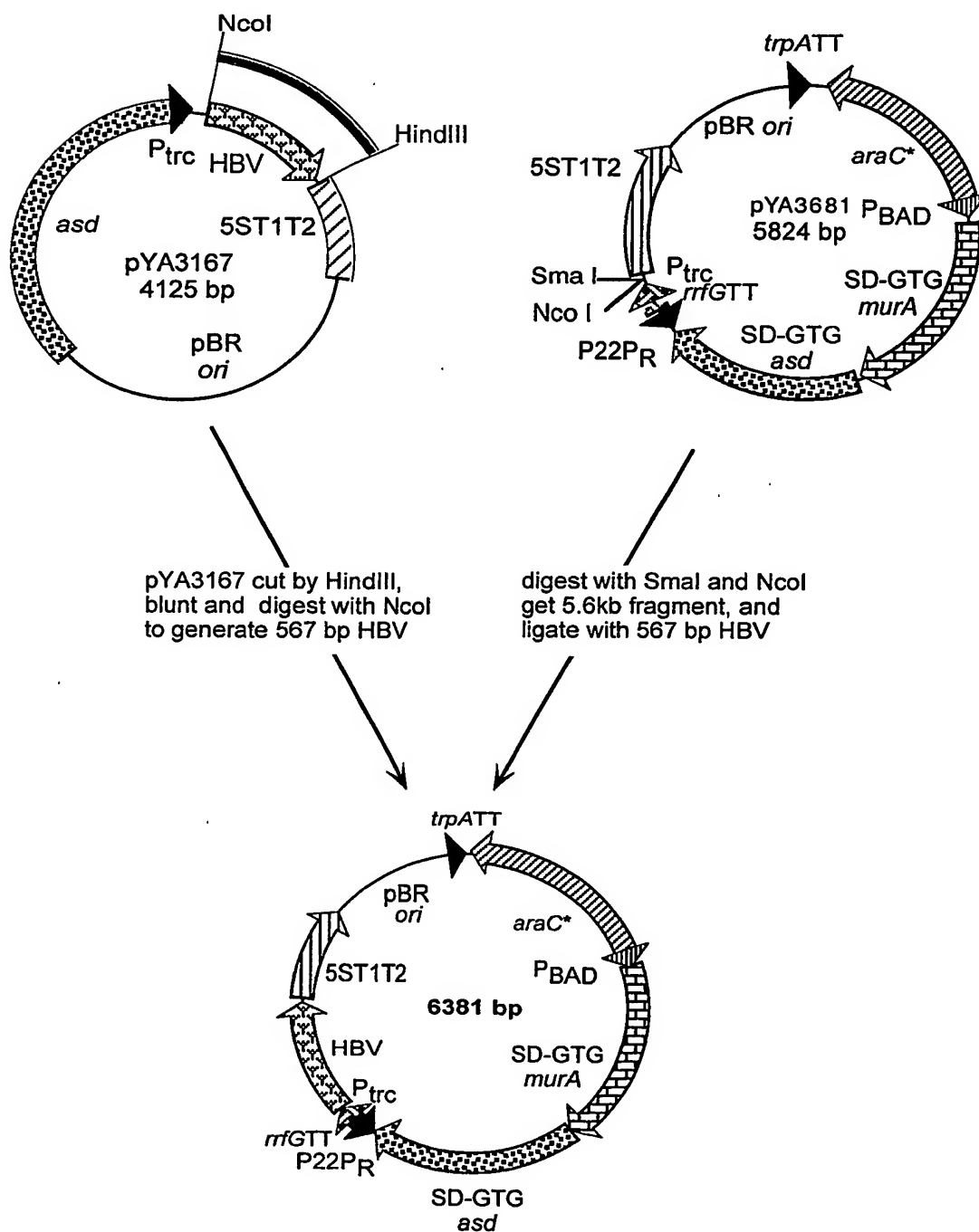


FIGURE 62. DNA and amino acid sequences of HBV core gene with preS1 and pre S2 epitopes in pYA3681

Pttrc promoter

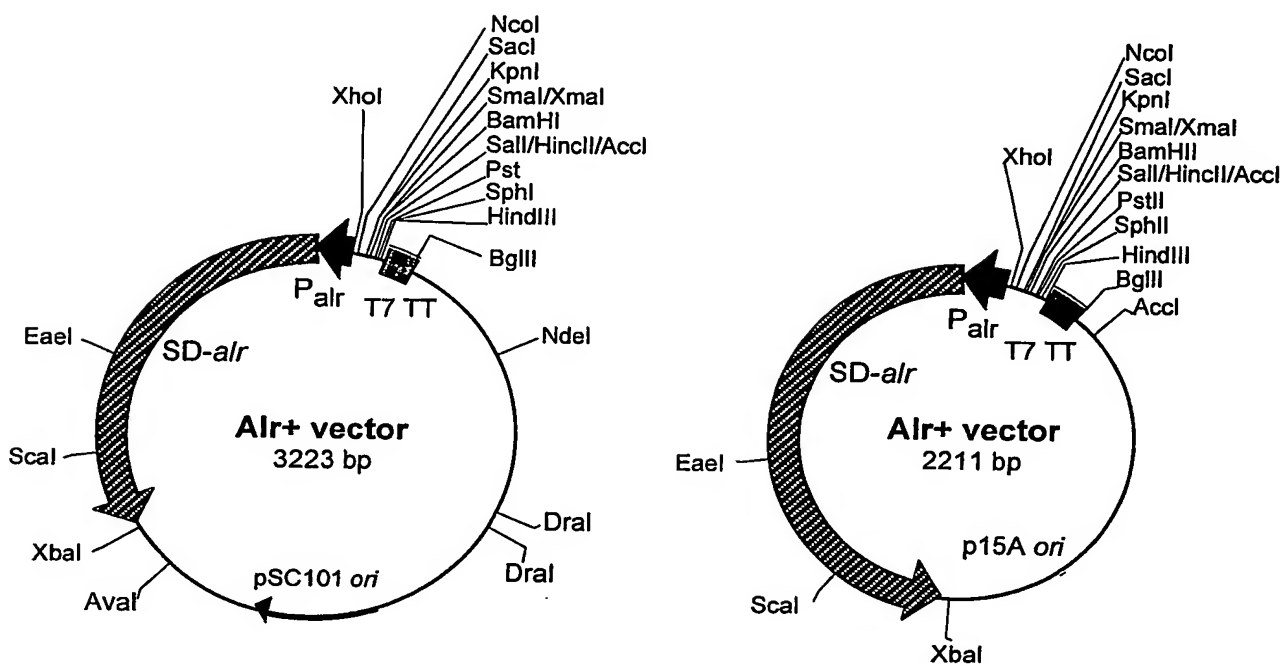
-35 -10
TTGACAATTA ATCATCCGGC TCGTATAATG TGTGGAATTG TGAGCCGATA ACAATTTTCAC

SD
ACAGGAAACA GACC

1/1	31/11
ATG GAC ATC GAC CCT TAT AAA GAA TTT GGA	GCT ACT GTG GAG TTA CTC TCG TTT TTG CCT
M D I D P Y K E F G	A T V E L L S F L P
61/21	91/31
TCT GAC TTC TTT CCT TCA GTA CGA GAT CTT	CTA GAT ACC GCC TCA GCT CTG TAT CGG GAA
S D F F P S V R D L	L D T A S A L Y R E
121/41	151/51
GCC TTA GAG TCT CCT GAG CAT TGT TCA CCT	CAC CAT ACT GCA CTC AGG CAA GCA ATT CTT
A L E S P E H C S P	H H T A L R Q A I L
181/61	211/71
TGC TGG GGG GAA CTA ATG ACT CTA GCT ACC	TGG GTG GGT GTT AAT ACT GCA AAT CCA GAT
C W G E L M T L A T	W V G V N T A N P D
241/81	271/91
TGG GAC TTC AAT CCC AAC AAG GAC ACC TGG	CCA GAC GCC AAC AAG GTA GGA GCT GGA GCA
W D F N P N K D T W	P D A N K V G A G A
301/101	331/111
TTC GGG TCT AGA GAC CTA GTA GTC AGT TAT	GTC AAC ACT AAT ATG GGC CTA AAG TTC AGG
F G S R D L V V S Y	V N T N M G L K F R
361/121	391/131
CAA CTC TTG TGG TTT CAC ATT TCT TGT CTC	ACT TTT GGA AGA GAA ACA GTT ATA GAG TAT
Q L L W F H I S C L	T F G R E T V I E Y
421/141	451/151
TTG GTG TCT TTC GGA GTG TGG ATT CGC ACT	CCT CCA GCT TAT AGA CCA CCA AAT GCC CCT
L V S F G V W I R T	P P A Y R P P N A P
481/161	511/171
ATC CTA TCA ACA CTT CCG GAG ACT ACT GTT	GTT AGA CGA CGA GGC AGG TCC CCT GGT GGC
I L S T L P E T T V	V R R R G R S P G G
541/181	
TCC AGT TCA GGA ACA GTA AAC CCT GTT	
S S S G T V N P V	

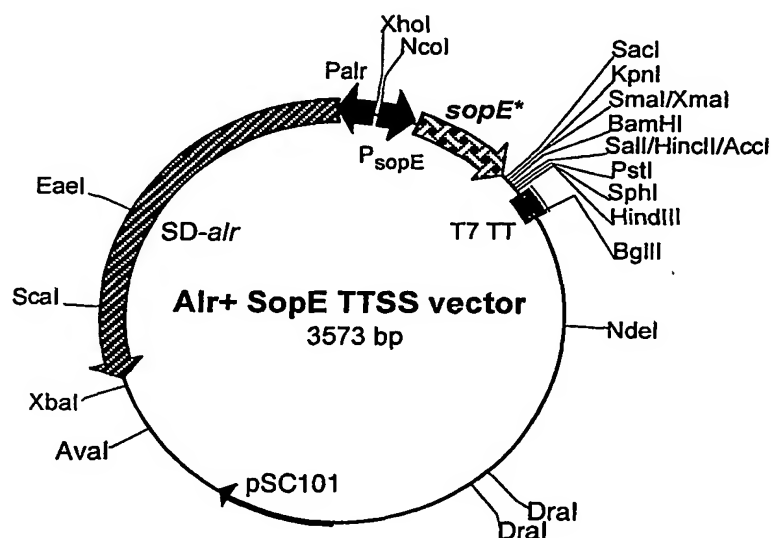
HBcAg (1-75)-pre-S(27-53)-HBcAg(81-156)-pre-S2(133-143)

FIGURE 63. Construction of Alr^+ plasmid vector with pSC101 *ori* and p15A *ori*



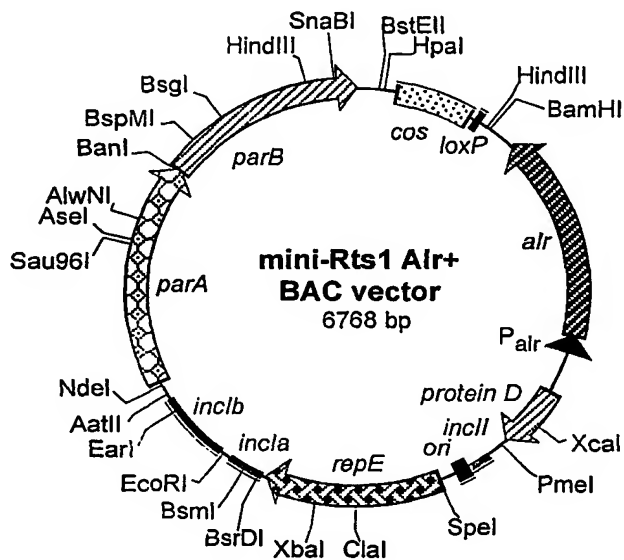
SD-*alr*: *alr*₋₁₀ to *alr*₁₀₈₀ + T
 P_{alr}: *alr*₋₇₆ to *alr*₋₁₁

FIGURE 64. Construction of Alr^+ plasmid vector with pSC101 *ori* to enable fusion of antigens with T-cell epitopes to the N-terminal end of the Type III effector SopE



sopE**: 342 bp of PCR product (*sopE*-93 to +249) including 93bp *sopE* promoter region and 249 bp of *sopE* from *Salmonella

FIGURE 65. Construction of BAC vector with *IncIa*, *IncIb*, *IncII* genes and *Alr*⁺ in place of antibiotic resistance genes



incII, *ori*, *repE*, *incIa*, *incIb*, *parA* and *parB* sequences are from Rts1 plasmid